PROGRESS OF SCIENCE IN INDIA

SECTION V

PHYSIOLOGY : PART I

[SUB-SECTIONS: PHYSIOLOGY AND BIOCHEMISTRY, PHARMACOLOGY AND VETERINARY SCIENCE]

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PROGRESS OF PHYSIOLOGY AND BIOCHEMISTRY FROM 1938 TO 1949

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INTRODUCTION

Research in Physiology and Biochemistry has been more comprehensive and more varied in this period than before and was not merely confined to the determination of physiological norms for developing a racial physiology as was alleged about the work of the preceding twentyfive years In fact, many findings point to the conclusion that the deviations in various physiological data for this country from European and American standards are chiefly due to poor dietetic conditions rather than to geographical peculiarities Hence it appears that some of the old and apparently well-established conclusions may have to be given up in the light of careful and prolonged researches done in this country The reader will find several such instances in the body of this review, but one or two glaring cases may be cited here. Thus, lathyrism which is believed to be due to the intake of *lathyrus sativus* (Khesari gram or dal) could not be induced in pups inspite of prolonged intake of a pure variety of this gram at 91 per cent. food level for a period of one year. Again angular stomatitis which is considered to be due to vitamin A deficiency, could be cured completely by gingelly oil containing 40 per cent linolenic acid, and not by vitamin A, or marmite, or skim milk No relation between the pre-test-dose level of vitamin B_1 in urine and the test-dose return or any correlation between its excretion and non-fat calories in the diet could be established. On the other hand, it was found that riboflavin excretion depends upon the protein and fat content of the diet and is proportional to carbohydrate fat ratio It was also noticed that vitamin D does not influence calcium absorption Further, no difference could be found in the digestibility and utilisability of animal and vegetable proteins. An oxalate and calcium rich food, like amaranth leaves, was found to maintain calcium balance in adult humans

It is worthwhile mentioning that several interesting new facts have also been brought to light by the work during this period Thus the specific dynamic action of proteins was shown to depend upon deamination and transamination processes. Injection of a large dose of vitamin B_1 may cause a marked depression of heart-beat. Ghee added at 8% level to the diet of rats was found to be superior to all other oils, hydrogenated or not, with regard to successful matings, shortness of gestation period, number of living young ones per litter, eye lesions etc. During germination of pulses *thiamine, ascorbic* acid and niacin increase enormously. The detection of the presence, in Bengal gram, of hesperidin, similar to vitamin P and essential for the growth of guineapigs is also notable. Seventytwo per cent rice in the diet

of rats causes fatty infiltration and vacuolation of liver cells. The isolation of neuro-toxin, cardio-toxin, haemolysin and choline esterase from snake venoms and the determination of their properties and in some cases the molecular weights deserve mention.

It will be deemed curious that investigations in Physiology have been mainly directed towards dietetics, nutrition and biochemistry to the neglect of its other branches. This is mainly due to the fact that the Government have been so long paying handsome grants to workers on these lines in view of the abnormally low nutritional status of the average people of this country. If physiology is to develop properly in all its branches and to play a useful and vital role in the defences of independent India, extensive researches on circulation, respiration, excretion, nerve-muscle physiology, nervous system and the sense-organs of eye and ear would be necessary to help the various units of the Defence Forces liable to be exposed to widely and quickly varying conditions of temperature, atmospheric pressure, illumination, external noise, physical strain and privation Such extensive researches would be possible only when properly trained personnel and fully developed and equipped laboratories are available. These conditions cannot possibly be realised unless the Central Government makes provision for the foundation of a Central Physiological Laboratory at a suitable place in India and for the training abroad of selected physiologists with an adequate knowledge of physics, chemistry and advanced experimental physiology This demand of physiology is too urgent, too pressing and too important to be deferred to a later period and it is hoped that this question would receive immediate attention

As the research work under review is quite extensive, particularly in the fields mentioned above, a brief survey of the important investigations is considered desirable and is given below. The author apologises to the workers concerned for any omissions. The survey is followed by abstracts of the classified literature and presented under fifteen heads, for ready reference.

Brief Survey of Research Work During the Period

1 *Blood* The various physiological data about plasma constituents and blood corpuscles have been collected in some provinces and their variations in diseases have also been noted. These data in some cases differ appreciably from European standards and also from province to province. How far these variations are due to racial characteristics or to persistently low dietetic standards requires to be ascertained. The blood groups of various communities in Calcutta have been studied and the presence of M and N factors amongst some Indians has also been ascertained The isolation of both acid and alkaline phosphatases from R B C. deserves mention, but their significance should be elucidated The prothrombin time of some healthy people has also been measured. The determination of 'pK.S P.' values of $CaHPO_4$ and $Ca_3(PO_4)_2$ promises to be of considerable value for detecting the onset of rickets in infants and children.

2. *Circulation* Electro-cardiograms of healthy persons showed a lower voltage of Q R.S. complex than those of Europeans The actions of some drugs on E C.G. have also been studied The action of drugs on heart explants from chick embryos has been investigated with a view to determining their effects on uninnervated or partially innervated heart muscle It has been shown that both adrenalin and acetyl-choline cause constriction of pulmonary vessels of various animals and that the effect of acetyl-choline is not potentiated by eserine.

3. *Respiration.* The vital capacity of students from several places has been studied and correlated with physical measurements.

4 *Enzymes and Digestion* The nature of the difference between thrombokinase and enterokinase has been studied The activity of the acid and alkaline phosphatases of R.B.C. has been estimated in different ionic solutions The activity of serum and tissue phosphatases under different conditions has also been investigated In burning feet syndrome which is considered to be due to pantothenic acid deficiency, *the activity of the enzyme acetylase which has pantothenic acid in its prosthetic* group is not always diminished

The digestive power of papain powder has been estimated Hunger contractions are found to be associated with increased acid secretion in the stomach, and they disappear after drinking 100 cc. water The digestibility of hydrogenated ground-nut oils of different melting points and containing different amounts of iso-oleic acid is the same.

5. *Absorption and Metabolism* The basal metabolic rate has been determined in different places and its variations with environmental temperature and humidity have been noted The low B.M R., due apparently to low thyroid activity, should be investigated in relation to the tyrosine content of our food for it was found that low B M R is not due to racial or climatic factors The determination of B M.R of the same person during different seasons in a period of 14 months shows that its variations are within 2 per cent of the average, being slightly higher in summer than during winter The variations of blood sugar in pregnancy and slow starvation and its relationship with phosphate in health and diabetes have been ascertained Olive oil and butter are more readily absorbed than mustard oil which is again more quickly absorbed than cocoa-nut oil from the rat's intestine, from human intestine butter, ghee and cocoa-nut oil are more rapidly absorbed than ground-nut or sesame oil. Ametin, a vegetable product, prevents diabetes caused by injection of di-acetic acid or β-hydroxy-butyric acid. The absorption of oil from the intestine is not affected by the degree of unsaturation, but thermal treatment reduces absorption by increasing the viscosity and thus interfering with its emulsification. The effect of some drugs on the absorption of fat and sugar has been studied Carotene in colloidal solution in water is absorbed from the intestine of rats in the presence in glucose solution is rapidly absorbed from the intestine and transformed into of only 0.04 per cent of fat In vitamin A depleted rats a suspension of carotene

vitamin A in the liver. No significant difference between digestibility and utilisability of vegetable and animal proteins could be detected in humans. The cause of alloxan hypoglycaemia has been investigated and different conclusions have been arrived at by different workers The specific dynamic action of amino-acids is found to be dependent on deamination and transamination processes. Thio-uracil depresses the specific dynamic action of glycine and glutamic acid but not of tyrosine.

6. *Food and Dietetics.* The nutritive values of vegetables, fish-liver oils, cereals and pulses and the biological values of fish proteins have been investigated. Parching of rice and pulses, drying at high temperature of fish proteins and addition of nicotinic acid to a diet increase their biological value. The minimum protein requirement on rice and wheat diets and the average protein requirement for maintenance have been ascertained. Appreciable supplementary relations exist between proteins of '*rubee*' fish and of '*khesari* and *musur*' (i.e. lentils) *dals* for the maintenance of nitrogen equilibrium The copper content of various foods and the fat content of various fishes have been determined (*hilsa* — 19.4 per cent, *Kai* and *Bhangar* — 8.8 per cent fat) Iron present in combination with fish proteins, is available after peptic digestion The percentage availability of Ca and P in rice and millets has been studied. Vitamin C, carotene, sugar and acidity of unripe and ripe mangoes of different varieties have been determined. Bengal gram contains hesperidin which is essential for growth of guineapigs and is similar to vitamin P. High pulse diet, alone or with cereals, damages liver and kidney, but such damage is prevented by green vegetables, butter, yeast etc. The cocoanut kernal has been analysed The effect of germination, autoclaving and heat processing on the digestibility and biological value of some '*dals*' has been noted Rats kept on 72 per cent rice diets develop fatty degeneration and vacuolation of cytoplasm of liver cells, due mainly to the deficiency of methionine in the diet Large amounts of Ca, P, and Fe are lost with the discarded rice-wash water.

7. *Vitamins:* The influence of Vitamin D on Ca and P absorption in rickets, on Ca and P content of soft tissues and bones, on the excretion of nitrogen, Ca and P and on the ionic products of Ca^{++} and $HPO_4^{=}$ in blood serum has been ascertained. Vitamin D does not influence Ca-absorption of healthy adults Vitamin B_1 first causes depression and then rise in rate and force of frog's heart-beat, but in humans it produces severe depression of heart-beat. According to another worker, vitamin B_1 in high concentration annuls to varying degrees the effects of acetylcholine in plain, skeletal and cardiac muscles. The percentage excretion of vitamin B_1 after a test dose has been estimated by various workers. A large dose of it is markedly diuretic In vitamin A deficiency the myelin sheath of rabbit's peripheral nerves degenerates. Vitamin A and Carotene of foods are not affected by boiling in open vessels, but are lost when butter is melted into ghee The vitamin A content of liver and body oils of some fishes has been determined The liver oils of some fishes are about 30 times richer in vitamin A than cod-liver oil; and

the body-oil of *Hilsa* does not contain vitamin A. A method of estimation of *niacin* alone or in presence of sugar has been reported The niacin content of wheat is higher than that of rice, and of rice than that of millets. Although poor maize diets contain more niacin than poor rice diets, the former is pellagragenic. Washing removes 60 per cent niacin from raw milled rice, but 12 per cent only from parboiled rice. Rats could synthesise niacin in their body During germination of pulses, the niacin content increases enormously mainly on the second day, the contents of vitamins B_1 and C increase mainly on the third day and the riboflavin content also increase It has been reported by some workers that dry skin and phrynoderma respond to treatment with vitamin A and not with linolenic acid, and angular stomatitis responds to yeast and not to niacin, while others have reported that gingelly oil containing 40 per cent linolenic acid alone can cure phrynoderma but not vitamin A, marmite, or skim milk, stomatitis caused by a milled rice diet is cured by eggs and yeast autoclaved in alkali Niacin present in blood is mostly concentrated in the red cells Chloretone and D-L-tryptophan enhance the formation of niacin by *Phaseolus mungo* during germination. Its trigonelline content diminishes simultaneously with the increase of niacin. Scrotal eczema could be cured by vitamin A and vitamin B_2 without naicin. The availability of thiamin, niacin and riboflavin present in cereals and pulses diminishes after cooking, and in fresh foods the availability of thiamin is higher than that of the other two vitamins On curdling milk, its niacin value is reduced by half after 72 hours, while its thiamine increases slightly and riboflavin more appreciably. An anti-B_1 factor is present in green gram, rice-polishings, ragi, carp muscle and viscera No relation could be established between urinary thiamine and its intake or between pre-test-dose level and test dose return between its excretion and non-fat calories in the diet A rapid chemical method for vitamin B estimation has been devised. Riboflavin excretion is influenced by protein and fat content of the diet and is proportional to the carbohydrate fat ratio Its microbial synthesis takes place in the intestine A bio-assay of pyridoxine based on the rate of growth of deficient rice-moth larvae has been described. The burning sensation of the extremities has been successfully treated with marmite and calcium pantothenate The vitamin A status of various people has been determined. Conjunctival pigmentation and Bitot's spots are not curable by vitamin A. Optimum and minimum requirements of vitamin A of persons of different ages have been determined. Large intake of vitamin A inhibits the secretion of the thyrotropic hormone of the anterior pituitary and thus decreases thyroid weight, neutralises thyroxine and reduces B M R The vitamin C content of various foods, herbs etc. has been assayed. There is a difference of opinion as to the suitability of Rotter's test for assaying vitamin C status. Vitamin C delays the onset of fatigue of skeletal muscle and augments the contractions of skeletal and cardiac muscles. It has no effect on gingivitis The growth of incisors of guinea-pigs is dependent upon their vitamin C status Mannose increases the vitamin C

content of legumes during germination. Vitamin C largely prevents, the rise of blood sugar after sugar ingestion, and hypo-vitaminosis is associated with ocular inflammation. In scorbutic guineapigs glucose tolerance, liver glycogen, and the insulin content of pancreas diminish appreciably The size of the adrenals and their adrenalin content increase in scorbutic guineapigs, but this increase in adrenalin is not responsible for lowered glucose tolerance The chloride content of blood does not change. The number of islets and their size increase, but β-cells are degranulated. Intake of 500 mg of vitamin C for 3 weeks makes fasting blood sugar normal in diabetes, improves sugar utilisation and prevents excretion of sugar in the urine. Gluco-ascorbic acid is not antagonistic to vitamin C.

8. *Nutrition:* The average daily calorie requirements for men and women of Bombay have been calculated A diet of Tapioca alone is fatal to rats, but with skim milk tapioca promotes growth The addition of calcium-lactate does not affect the biological value of proteins but increases the growth of children. The daily requirements of Ca and P have been ascertained. Typical vegetarian rice diets can maintain phosphorus balance but not calcium balance, but wheat diets can do both. Different Ca: P ratios in the diet do not influence the Ca and P content of soft tissues The Ca content of tissues in rickets and hyper-vitaminosis D has been studied. The iron present in leafy vegetables, spices and condiments is of low availability but '*Palta*' is rich in available Fe Phosporus present in cereals and pulses is largely phytin — P (50 to 70 per cent) which is practically absent in vegetables. Phytin increases on soaking seeds in water. On the ripening of grains proteins of mixed vegetarian diet can maintain N-equilibrium of adult rats, but for good growth of young rats a small amount of milk or animal protein is necessary. Carbohydrates of all pulses excepting Bengal gram and soya bean are nearly completely available. The daily adult requirement of magnesium is 0 43 g which is readily obtained from vegetables and legumes. Calcium absorption from the intestine takes place through both blood vessels and lymph vessels. It is available from betel leaves taken with lime and also from bones of small fish. Vitamin D has no influence on its absorption. Calcium utilisation, i e $\frac{\text{Ca-retention}}{\text{Ca-intake}}$ is 0.87 for skim milk, 0.82 for cabbage, and 0 70 for lady's fingers or drums-stick in the case of young rats. The anterior pituitary extract causes an increase in calcium retention. All these vegetables and even amaranth, rich in oxalate, have a favourable effect on the Ca balance of adult humans. Food cooked in tinned vessels retards growth of rats. If the fluoride content of water exceeds 1 ppm, mottled enamel occurs, but a liberal supply of vitamin C prevents it to some extent. The biological values and digestibilities of proteins of mixed rice and mixed wheat diets are 60 and 80 and 59 and 89 5 respectively Proteins of goat's milk have a biological value inferior to those of cow's or buffalo's milk for the growth of rats. Casein of ass' milk has more P and arginine-N than that of the other milks, and lactalbumin from ass' milk has more lysine but less tryptophane, tyrosine and cystine than that of the

other milks For maintenance, Cow's milk proteins have a greater biological value than those of goat's or buffalo's but their digestibility is the same. The biological value of proteins of mixtures of rice and pulse increases when pulse is substituted by milk. With regard to growth rate of rats, cow-butter, buffalo-butter, ground-nut oil, mustard oil, cocoa-nut oil, sesame oil, soya-bean milk proteins are 90 per cent as efficient for haemopoiesis as casein; and soya-bean milk curd-proteins are as well utilised as cow's milk curd-proteins Rats retain 82 per cent Ca and 87 per cent P, when fed with soya-bean milk enriched with calcium phosphate Soya milk causes normal growth of infants without any digestive trouble Ca and P of soya milk and cow's milk are equally well utilised, but the utilisation of the proteins of the former is 86 per cent of that of the latter. Feeding hydrogenated ground-nut oil to rats causes an increase of cholesterol in liver and brain, of phospho-lipid in brain and of total fatty acid in liver The importance of addition of food yeast, ground-nuts, skim milk or some pulses to proor rice diet has been ascertained in relation to growth In growth experiments with rats, given a basal diet, the addition of 8 per cent ghee is superior to that of all other oils, hydrogenated or not, with regard to successful matings, shortness of gestation period, number of living young ones per litter, haemhorrhages at the angles of eyes and opacity of the cornea *'Khesary dal'* at the level of 91 per cent of a diet given to 4 pups, caused mild symptoms of stiffness or parosis of hind legs in only 2 animals, these symptoms became milder with continued feeding for a year Hen's egg-white is much superior at 8 4 per cent protein intake to duck's egg-white for the promotion of growth, due to higher digestibility and the histidine content of the former being nearly double that of the latter, but at 25 per cent protein intake the differences in growth are slight.

9. *Biochemistry.* The affinity of γ-globulins for H^+ ion is less than that of other serum globulins. Preparation of peptone for bacteriological work has been carried out using papain An accurate method for the estimation of ammonia nitrogen in biological materials in the presence of protein has been devised The estimation of niacin precursor has been done by a modified method. The reducing substances in the ejaculated semen of some animals have been analysed Fructolysis in semen is proportional to sperm concentration The influence of thyro-protein feeding on sperm concentration has been noted The action of trypsin on insulin is inhibited by the presence of proteins which are easily digested by trypsin The increase in serum phosphatase in pulmonary tuberculosis is prevented by the intake of large doses of vitamin C. The choline-esterase of cobra venom has been isolated and its properties studied. Crystalline haemolysin from cobra venom has also been isolated. The effect of various substances on its activity, its reversible inactivation, its various properties, molecular weight and chemical composition have been studied. Some amino-acids in cobra-neurotoxin have been estimated Effects of snake venoms on the oxidation of glucose and its metabolites and the cyto-chrome and cyto-chrome-oxidase system have been studied. Proteins of rattle-snake venom

and active principles from the venoms of *Bungaras fasciatus* and *Viperi russellii* have been isolated. Cobra neurotoxin has been separated The reaction between Russel's viper venom and its anti-venine has been studied and the coagulating principle of this venom has been separated. The effect of cobra venom on the synthesis of acetyl-choline by the brain cells of rats and pigeons has been studied. Cardiotoxin has been separated, its molecular weight has been determined and the effect of temperature on its stability studied. The effect of different absorbents on the toxicity of cobra venom has been studied. The conversion of tyrosine into thyroxine depends on its specific linkage in the protein molecule The incorporation of methionine into tissue protein is retarded when it is required to be converted into cystine.

10. *Hormones and Endocrine organs:* Artosterone has been shown to be highly androgenic. An enzymic method for the estimation of adrenalin has been developed. Fatty infiltration of liver caused by anterior pituitary extract is not prevented by choline chloride On a poor rice diet parathyroids of rats are hypertrophied, and there is an increase in peri-vascular connective tissue. Milk secretion of cows under the influence of various hormones has been studied Growth of animals under the influence of various hormones has been investigated. Influence of stilbestrol on blood sugar and N P N. and on vitamin C and cholesterol content of adrenals has been studied. The nature of action of various goitrogens has been indicated.

11. *Excretory organs·* During slow starvation kidney may develop mild nephritis. The normal urinary constituents of Oriyas and Bengalees have been analysed and have been found to differ from those of Europeans. The average chemical composition of the sweat of Indians has been determined Urinary porphyrin excretion in stomatitis is normal The maximum and standard urea clearance of Indians are 44 cc and 33 8 cc i e lower than the European averages. N-retention of the body is higher with the intake of vegetable proteins than with that of animal proteins In Hyderabad (Deccan) the maximum urea-clearance is 70 to 75 per cent of American standards.

12. *Nerve and Muscle Physiology·* The action of varying doses of adrenalin and the optimal temperature for the maximum action of acetylcholine and adrenalin have been studied Rheobase and chronaxie were determined in vitamin A and vitamin B_1 deficient animals and in normal and under-fed persons. The action of vagus or acetylcholine on isolated strips of dog's stomach having peri-arterial sympathetic innervation has been studied Ephedrine potentiates adrenalin action on rabbit's gut The phasic and tonic contractions of unstriated muscle are probably caused in different ways by the stimulation of attached nerves, the former by a liberated chemical at the neuro muscular junction and the latter by the action potential. There are two kinds of tonus in the unstriated muscle, one being controlled by the chemical stores in the muscle and the other by the muscle substance itself. Adrenalin and acetylcholine act directly on the muscle and also indirectly through nerves.

13. *Miscellaneous:* Complete medical studies were made of 407 cases of slow starvation and of diseases that occur in such cases. Biochemical changes in blood in liver cirrhosis were studied. Chemical changes in blood and urine in cases of heat stroke were noted. The distribution of lead in various tissues of the body has been ascertained. The extent of elimination of chloral unconjugated with glucuronic acid after administration of chloral hydrate may be used as a measure of liver damage in humans. The difference in osmotic pressure between egg-white and yolk is not due to Donnan Membrane equilibrium, but is the result of a dynamic state due to vitality of the vitellin membrane. Food may be contaminated with lead through the use of solder or enamel. Thirty or more Bodansky units of phosphatase in serum are a sure indication of biliary obstruction Neo-stibosan is non-toxic but urea-stibamine is toxic to frog's heart at a dose corresponding to the initial human dose. The effects of histamine on frog's heart have been studied. *Brihat Kasturi-Bhusan* is a good stimulant to heart. A method for tracing uniform genesis of tetanus curves has been devised. Infiltration of 5 per cent emulsion of Lifebuoy carbolic soap at the site of bite of cobra or krait has been described as a temporary life-saving measure. Permanganate is valueless and may be even harmful Biochemical characteristics of cobra and Russel's viper venoms have been studied Studies have been made of the action of neurotoxin, haemolysin and cholinesterase isolated from cobra-venom on heart, blood-pressure and respiration The action of cobra venom and cardiotoxin on toad's heart and voluntary muscles have been investigated in comparison with those of Ca, Na, K, acetyl-choline, digitalis, saponin and stropanthin. Nutritional oedema may be caused by some anti-diuretic factor which is present in the urine of such patients, besides the fall in the serum albumin level of blood.

Blood

1 The biochemical picture of blood is altered significantly with regard to the following in cases of slow starvation

(a) Lowered sugar content without symptoms of hypoglycaemia,

(b) Non-utilisation of glucose by the body, as proved by the persistence of high sugar content of blood for 4 hours after sugar intake, as is done in sugar tolerance test Absorption of sugar from the gut is not materially changed,

(c) Reduction of total plasma protein and alteration of albumin-globulin ratio, amounting to even reversal of the same,

(d) Average Hb content — 1 74 g per 100 cc

2. In a case of prolonged starvation the blood sugar was 17 6 mg per cent without any hypoglycaemic symptoms (Chakravarti, 1945).

3. Blood norms with regard to urea, N.P.N., creatinine, uric acid, chlorides and cholesterol of Bengalees, their mean values and standard deviations have been determined (Chakravarti, 1945).

4. Banerji, Tej and Senapati (1949) found by investigating R B C. count, total and differential count of W B C., blood sedimentation rate, haematocrite value, mean cell-haemoglobin and mean cell volume of 50 apparently healthy individuals of both sexes at Cuttack, that high sedimentation rate, slight macrocytic anaemia, and eosinophilia occured in many of these examined persons without clinical symptoms

5 Mean red cell diameter of Bengalees — 7,288 μ (= 7,202 μ — Price Jones). Range of variation — 6.644 μ to 7.932 μ (= 6 686 μ to 7 718 μ — Price Jones). (Napier, Sankaran, Swaroop and Rao, 1939).

6 Choline-esterase activity of blood and serum of different animals under different conditions was studied and it was found to be the same with the arterial and venous blood and serum; It can be stored in refrigerator without loss. Sleeplessness change in environmental temperature, fasting or glucose feeding does not affect it (Mahal, 1938)

7. Sokhey, Gokhale, Malandkar and Billimoria (1938) examined the blood of 101 healthy Bombay women of 16-30 years age and found the following averages. R.B C. — 4 47 millions, Hb — 12.99 g, Cell-volume — 36 27 cc./100 cc (in oxalate solution), Hb-coefficient — 14 55 g/100 cc, volume-coefficient — 40 61 cc./100 cc., Color-index — 1.00, volume-index — 1 00.

8 Sankaran and Rajagopal (1938) estimated the Hb content of the girls of South India. This could be raised by administration of $FeSO_4$, the rise lasting for about 2 months

9. Glycolysis in normal blood is 6 05 mg per cent/hr. and in diabetic blood is 11 06 mg per cent/hr , but the rate of glycolysis in normal blood is 23 8 per cent less than that in diabetic blood (De and Bhattacharjee, 1938).

10. The mean R B.C diameter is 6.85 μ, i e., 0.28 μ less than that of Europeans (Rao and Rao, 1942).

11. Hb in pregnant women — 15 52 g/100 cc. and in non-pregnant women — 15 81 g/100 cc (Rao, 1938).

12. Non-protein nitrogenous constituents in mg per cent of blood of Indians : N.P N — 18.43 $\pm$ 1 37, Urea N_2 — 12.50 $\pm$ 1 34, Uric acid — 3 02 $\pm\frac{0\ 395}{3.02}$ *Creatinine — 1.17* $\pm$ *0.109*, Total creatinine — *1.45* $\pm$ *0 179*, *Amino* N_2 — 3.88 $\pm$ 0.437 (Gokhale, 1939)

13. After injection of penta-nucleotide, Na-Nucleinate, liver extract, histamine, adrenaline, and acetyl-choline, there is leucocytosis due to re-distribution of circulating W.B C's and mobilization of preformed reserves in bone-marrow (Dasgupta, 1939).

14. After examining 300 Indians it was found that M and N factors of blood are present in the following percentages: M —42.7 per cent, N — 10 7 per cent, MN — 46.7 per cent (Greval, Chandra and Woodhead, 1939).

15. Blood groups of various communities of Calcutta were studied (Greval and Chandra).

16. Hb of boys at 5 years — 11.5 g per cent and at 16 years — 13.5 g per cent Hb curve of females differs from that of males on the 11th year, and rises thence slowly to 12.5 g per cent. There is little difference between the Hb curves of Bengalee and Sikh boys (Napier and Dasgupta, 1940)

17 Iso-haemagglutination in blood has been reviewed (Greval, Chandra and Woodhead, 1941)

18. Bengalee women have lower Hb content and lower R B C. sedimentation rate than other Indian women (Napier, Neab Edwards and Das Gupta, 1941)

19. There is an important antigenic difference between haemagglutinogens M and N, for it is easier to produce an anti-O N serum and later by adsorption an anti-N-fluid than an anti-O M serum and anti-M-fluid (Greval and Chandra, 1941).

20. By projecting images of R B.C over a ground-glass screen so as to obtain 2000 times magnification and then measuring these images by super-imposing a celluloid protactor on which circles with diameters varying in lengths equivalent to 0.25 μ have been drawn with a fine pair of dividers, it is found that the maximum and minimum diametres are higher than those of Price-Jones' curve (Napier, Sen Gupta and Chandrasekhar, 1941)

21. Temperature and potassium-oxalate increase the rate of glycolysis in blood, but sodium-fluoride decreases it (Bose and De, 1942)

22. For the detection of pro-thrombin time, the stock solution of 1 in 20,000 Russel's Viper venom in water or in 0.025 M $CaCl_2$ sol kept under toluol, is quite stable (Iyengar, Sehra and Mukerji, 1942).

23 There are more of O and B blood-groups amongst Madrassis on account of Dravidian and Aryan mixture (Seshadrinathan and Timothy, 1942)

24 Surface tension plays a secondary part in initiating the process of haemolysis. Sometimes its increase tends to retard haemolysis, and sometimes facilitates it (Roy, 1943).

25. *Cholesterol does not retard ordinary haemolysis but in large dose retards* saponin haemolysis (Roy and Biswas, 1943)

26 Spectra of haemochromogen and cyan-haemochromogen have been described (Greval, Roy Chowdhuri and Das, 1954)

27. The composition of serum fatty acids is influenced by various other factors in addition to dietary fat level (Nhavi and Patwardhan, 1946).

28. If white rats are fed on a diet free from Vit K and 1 per cent sulphathiazole for 3 weeks, then there are (a) severe hypoprothrombinemia which may be

prevented by addition of vitamin K to the diet and (b) fatty change in liver (Braganca and Roy, 1947).

29. Plasma proteins in healthy individuals were fractionated by the method of Howe and the following results were obtained (values in g/100 cc)

Total Protein — 7.0 to 7 95.

Average for males — 7 51, and for females — 7 49

Albumin — 4.3 to 5 34, average — 4 93 for males

„ — 4.87 for females.

Globulin — 2 12 to 2.9, average — 2 58 for males

„ — 2 6 for females

Eu-globulin — 0 25 to 0 78, average — 0.55

Pseudo-globulin — 1 3 to 2.24, average — 1.84 for males and

1 74 for females

Fibrinogen — 0 12 to 0 48, average — 0.25

No significant difference in these fractions between the sexes or between vegetarians and non-vegetarians was noticed (Datta and Chakravarti, 1947)

30. Hyperglobinemia is associated with increase in both eu-globulin and pseudo-globulin fractions. When the total globulin rises above 5 g/100 cc, the increment is mainly due to eu-globulin fraction, as in cirrhosis of liver or Kala-azar (Datta, 1947*a*).

31. Increase in Vittamin A in blood leads to (a) a decrease in total amount of W.B.C., (b) an increase in lymphocytes and macrocytes, (c) a decrease in neutrophils and eosinophils, and (d) a shifting to right of Arneth and Schilling haemograms. Diminution of Vitamin A content of blood leads to physiological leucocytosis. Thus Vitamin A is related to the general defence mechanisms of the body (Hussan, Ibrahim and Khanna, 1948).

32. Both acid and alkaline phosphatases have been isolated from R.B.C (Ranganathan and Patwardhan, 1949).

33. Heparin can effectively counteract the effects of Russel Viper's venom *in vitro* and *in vivo* (Ahuja, Brooks, Veeraraghavan and Menon, 1946).

34 Lecithin and haemolysis have been discussed (Roy and Chopra, 1941).

35. Some observations were made on the lipoid content of blood in epidemic dropsy (Chopra, Majumdar and Roy, 1940).

36. Studies on haemolysis (a) There is no significant correlation between the fragility of human R.B C and such factors as mean corpuscular size, thickness, volume, Hb content and saturation, R B C. count etc. (b) Mean size of R.B C. (from 131 persons of age range between 18 and 25) as determined by direct Halometer — 7.021 $\mu \pm$ 0 019 (range — 6.4 μ to 7 75 μ). The standard

deviation and the c.v. per cent are 0 318 ± 0 013 and 4.533 ± 0.1893 respectively (Telang, 1940 and 1942)

37. Variations of plasma proteins in health and various diseases have been discussed (Dutta, 1948).

38. Clinical significance of hypoproteinaemia (Dutta, 1947).

39. *Hypoproteinaemia in surgical cases and its mode of action (Dutta, 1947)*

40. Prothrombin time in normal Indians (Braganca and Rao, 1946).

41. The average weighted mean of the polynuclear counts of 120 males in Hyderabad-Deccan (Age — 15 to 45) is 2,432 with a standard deviation of 0 234 This is much less than Crooke-Ponder's average for Europeans, although the total leucocyte count does not differ much The lower neutrophil count is compensated by higher lymphocyte and eosinophil counts (Rahman and Zeidi, 1941)

42 Ultra-violet irradiation stimulates the haemotopoietic system slowly by increasing the red cell count and the mean Hb conent, the leucocytes and the platelets. There is also some reticulocytic response. Infra-red rays produce a similar but less marked effect (Pal, Saha and Banerji, 1949).

43. (a) Moderate excess of $CaCl_2$ inhibits prothrombin time (b) Prothrombin time, determined by Quick's method, is found to vary between 15 and 20 sec in healthy adults in Vizag. In obstructive jaundice and cirrhosis of liver it increases to about 45 sec (Reddy Venkataramaiah, 1941 *a*, *b*).

44 Ponder's physical method for determining percentage haemolysis of blood has been found to be impracticable, but the application of Carey-Foster's principle gives better results. Selenium cell, on account of its lag, is found to be unsuitable for such experiments (Basu and Datta, 1937).

45. Ayapanin, a new haemostatic agent, has been isolated from 'Ayapan' leaves (Bose and Sen, 1941).

46 Ca, Mg and P content of serum, plasma and whole blood of normal, diabetic and tubercular persons were determined The inorganic and total acid-soluble P of blood is lower than normal in tuberculosis, but serum Ca and blood Mg remain normal (Roy, 1942)

47 Results of haematological examination of 81 healthy adults of Nilgiri are given (Coonoor Nut Res. Lab. Report 1949-50).

48. After examining blood serum of 79 cases of rickets in infants as children for total Ca, inorganic P and total protein and also that of 11 normal children for the same, and then calculating therefrom the concentration of (Ca^{++}) according to Maclean and Hastings (1935), of $(HPO_4 =)$ and $(PO_4 =)$ at pH 7.4 (which does not show any change before the onset, during the development or on cure of rickets) according to Sendroy and Hastings (1926), it was found that pK s p values of $CaHPO_4$ (i.e., the negative logarithm of (Ca^{++}) ×

(HPO_4 =) in rachitic cases were over 5.7 in 77 out of 79 cases, whereas in only one out of 11 non-rachitic children was the value above 5.7. Similarly with regard to pK s.p. values of $Ca_3(PO_4)_2$ (i.e., the negative logarithm of $(Ca^{++})^3 \times (PO_4\ \ )^2$), 77 out of 79 cases had values above 23 0, showing under-saturation, whereas in 2 cases out of 11 non-rachitic children the blood was under-saturated with respect to this latter salt (Patwardhan, Chitra and Sukhatankar, 1944).

49. Non-haem Fe and Cu increase significantly in blood in tuberculosis and the new-born child The total Fe of blood falls much below normal in diabetes and during pregnancy (Roy, 1939-41).

50 Alloxan interferes with the formation of prothrombin by liver and thereby causes hypoprothrombinaemia (Sen Gupta and Sen, 1949)

CIRCULATION

1. Banerji, Tej and Senapati (1950) after taking E C G. tracings with G.E C apparatus and Philips Cardiotron (both of which gave identical curves for the same person) of 50 healthy young men at Cuttack of the average age of 19, found that their average heart rate is higher and the voltage of Q R S. complex is lower than European or American standards

2. They also found that the following preparation gave as satisfactory results as the imported electrode jelly for E C G. tracings

Ordinary adhesive paste (Malay) + glycerine in equal parts + NaCl added to make a 9 per cent solution

3. Stimulation of stellate ganglion or injection of adrenalin causes vaso-constriction which is abolished by ergotamine. Stimulation of cervical vagi or injection of acetyl-choline causes vasco-constriction which is abolished by atropine and is not enhanced by eserine (Sinha, 1942 *a*).

4 Neurotoxin isolated from cobra venom augments heart-beat but paralyses respiratory movements (Sarkar, Maitra and Ghosh, 1942).

5. Factors in the maintenance of normal blood pressure of humans have been discussed (Basu, 1948).

6. *Autonomic nerves have a role in controlling circulation in the systemic* blood vessels of frog (Roy and Gupta, 1949).

7. The action of certain cardiac drugs on embryonic heart explants has been described (Chopra, Das and Mukherji, 1938).

Heart explants from chick embryos, 2 to 7 days old, were cultivated in vitro. Cardiac nerves do not develop at this stage. Lenadigin, thevetin and strophathin are predominantly inhibitory to such aneural cardiac musculature, while caffeine and cardiazol are predominantly stimulant. Caffeine causes the pulsations of heart explants to continue for a longer period than the controls.

8. Antagonism between ergotamine and adrenaline has been discussed (Narayana, 1938).

9. Vaso-motor responses in the isolated perfused guinea-pig's lungs have been recorded (Sinha, 1942 *a*).

10. Observations on the behaviour of pulmonary vessels in the isolated, perfused rat's lung were made (Sinha, 1942 *b*).

11. Nervous and chemical control of pulmonary vessels of the frog has been studied (Sinha, 1944).

12. Frog's heart perfused with, or hearts of anaesthetized rabbits, cats, and dogs infused intravenously with 1 20 Tinct. Digitalis solution show the following characteristics in E C G.: S-T interval is first depressed and is then later followed by an infarct type of Q R.S. complex. T-wave is more erect and pointed

13. E C G of 75 men at Hyderabad-Deccan was found to be similar to those in U S.A.

14. Role of K in some irregularities of perfused heart (Benwari, 1949).

15. Some sulphonamides have a temporary stimulating action on heart, but on prolonged use all of them cause irregularity in heart beats, or grouped beats, extreme slowing and sometimes complete stoppage, through their action partly on autonomic ganglia but mainly on cardiac muscle. In small doses they have no effect on carotid pressure, but in bigger doses produce a fall therein Sodium bicarbonate or acetate can sometimes remove their toxic effects, and if administered simultaneously with sulphonamides can improve the heart action (Pal, 1947)

16 Moot Mekesson Cardiac index was determined in 244 medical students of Vizag Eighty per cent of these had an index between 34 and 80 per cent and thus have fair risks for surgical procedures, 10 per cent have an index between either 25 to 33 per cent or 81 to 100 per cent and therefore have poor risks for operation, and the remaining 4 per cent — an index either above 100 per cent or below 25 per cent and are therefore unfit for operation (Reddy and Sitaramayya, 1948).

RESPIRATION

1 Average vital capacity of Vizag boys is found to be 2985 cc with Sanborn's Spirometer (Wet Type) By correlating vital capacity with height, weight etc., it was found that the highest correlation was obtained with sitting height and vital capacity (Reddy and Sastry, 1944).

2. After the administration of barium meal there is respiratory dyspnoea (Chopra and Chakravarty, 1940).

3. Studies in vital capacity of Bombay Medical students were made with reference to statistical correlation with physical measurements (Telang and Bhagwat, 1941).

4. *Intravenous injection of nicotine into rabbits and dogs produces apnoea at*

the end of full inspiration in rabbits and at the end of expiration in dogs. It is followed by hyperpnoea in both cases (Rahman and Abhyankar, 1942).

5. With windows fully opened out and fans moving, the air temperature and dry kata cooling power of 2nd class compartments in Railways between Madras and Waltair indicate oppressive discomfort during summer (Reddy, 1947).

6. A study by manometric method of the inhibition of respiration of muscle, brain, liver and kidney by KCN, malonate, and arsenite was made, and the mechanism of tissue respiration was discussed in the light of these observations (Roy and Das, 1942-43).

ENZYMES

1 Giri and Showrie showed that rats fed on poor South Indian diet have a higher serum-phosphatase activity than when the diet was supplemented with calcium lactate. Addition of fat increases serum phosphatase activity (Giri and Showrie, 1939).

2. Enterokinase is a mixture of a kinase which is similar to blood-kinases and another factor which can activate trypsinogen. It thus differs from thrombokinase (Iyengar, 1942)

3 Starvation decreases the alkaline phosphatase content of different tissues to different degrees It is regenerated in some tissues by some dietetic factors which are inactive in the case of other tissues (Roy and Sen, 1944 *a*).

4 An alkaline phosphatase has been detected in R B.C and has been separated from its acid phosphatase which is far more active. Alkaline phosphatase of R.B C. is weaker than that of plasma and is inhibited by $ZnSO_4$, NaCN and NaF at M/200, M/200 and 5/50 concentrations respectively. This phosphatase is activable by Mg^{++}. In rachitic rats there is decrease in plasma alkaline phosphatase, but not in acid phosphatase (Conoor Nut. Res. Lab. Rep. 1947-48 and 1948-49).

5. The enzyme, acetylase, which has pantothenic acid in its prosthetic group, brings about acetylation of sulfanilamide and choline in the body and is probably also responsible for acetylation of para-amino-benzoic acid (P.A B.) as it is affected *in pantothenic acid deficiency. Since 'burning feet syndrome' is a manifestation* of pantothenic acid deficiency (according to Gopalan), normal persons and persons suffering from the syndrome were given 100-200 mg of P.A.B. and the acetylation value of P.A.B. excreted in urine was determined Then pantothenol in the dose of 350 — 750 mg was given to both groups along with 100 — 200 mg of P A B. and the acetylation value was again determined. It was found that the suffering persons had a distinctly lower acetylation value which improved markedly after pantothenol intake. But there were several cases of 'burning feet', who had normal acetylation values and did not improve after pantothenol intake This shows that there are also other factors involved in 'burning feet' syndrome (Conoor Nut. Res. Lab. Rep. 1948-49).

6. During scurvy phosphatase activity is markedly lowered in serum and bones, but is not affected in other tissues (Roy and Sen, 1944 *b*).

DIGESTION

1. The isolated stomach mucous membrance of frog, mounted in an improvised single chamber and nourished by a fluid simulating frog's plasma, secretes HCl, the maximum amount with a pH of 2 8 being obtained in 3 to 4 hrs The secretion is inhibited more by changes in K + and Ca + + than by HCO_3, PO_4 and Mg + + and this sensitivity to ionic changes is nearly abolished by histamine (Venkataramia, Thesis for M.Sc. of Madras University).

2. In determining the digestibility coefficient of proteins of food, it is necessary to determine the N P.N of the food and the protein content of the food separately, as the N P.N. may be poorly available in digestion. If the protein content of a food is obtained by its N_2 content multiplied by 6 25 and then its digestibility is ascertained, it is likely to be under-estimated on account of the above reason (Swaminathan, 1938).

3 With alcoholic test meal the fractional gastric analysis shows that (a) there is no difference between Hindus and Moslems with regard to HCl secretion in stomach (b) that hyperchlorydria is more common amongst men, and (c) that the percentage of onset of hyperchlorhydria increases with age (Mangalik, Goel and Mangalik, 1942).

4 The digestibility of hydrogenated ground-nut oil of melting-point at 29 5°C does not differ significantly from that of one of melting-point 40°C (Misra and Patwardhan, 1948).

5 300 g of mutton mince when mixed with 5 g of papain powder was found to be completely digested after 2 hrs. at 60°C, as estimated by formol-titration, oxidisable matter content or total protein content Of these 3 methods for determining the course of digestion, formol-titration is the simplest With half the quantity of papain, the digestion is delayed by 1 hr (Bose, 1939)

6. Gastric hunger contractions in a human subject do not depend either on lowering or rise of blood sugar They are also not affected by the injection of *pituitrin, or ephedrine or by tightening of abdominal belt*, but disappear after the drinking of 100 cc. of water (Rahman, 1943).

7 By introducing two tubes into the stomach of a man, to one of which a rubber balloon is attached and the other is used for aspirating the gastric juice, it is found that hunger contractions are associated with increased acid secretion (Rahman and Abhyankar, 1944).

8. Potato meal has been used in the study of gastric secretion (Benawri, 1946).

9. No significant difference in digestibility was observed between ground-nut

oil and ground-nut oils containing 9 per cent and 19 per cent iso-oleic acid (Conoor Nut. Res. Lab. Rep. 1947-48).

Absorption and Metabolism

1. Batliwala (1947, 1948 *a*, *b*) investigated the incidence and causation of glycosuria in pregnancy.

2. Banerji, Subrahmanyam and Tej (1951) determined B M.R. of 22 normal students of Cuttack Medical College and found that the B.M.R. of 6 deviated by more than 10 per cent from accepted standards, although the average B M.R of all the students differed by only 3 per cent and the average B M.R of the remaining 16 by 4.4 per cent from the Mayo foundation normal standards They also pointed out that B.M.R. determination by Read's formula gives unreliable figures.

3. After glucose ingestion, blood phosphate decreases for 1 hour and then begins to rise, reaching the normal value of 3 5 mg per cent at the end of 2 hours. In diabetes the blood phosphate change varies with the severity of the disease. Insulin accelerates the decrease of blood phosphate by releasing it from the circulating blood It is likely to play a part in the phosphorylation of glucose (Bose and De, 1939).

4. During transition from cool to hot, humid tropical climate B M R. and oral temperature rise slightly on the first two days. From the 3rd day the B M.R. begins to fall and at the end of a week it becomes lower by 10 per cent The adjustment is brought about by a diminution in the activity of the thyroid (Mason, 1944).

5 Rats were fed various fats and oils by stomach tube. They were killed, at the end of 2, 4 or 6 hours Their gastro-intestinal tract was then removed and the fat or oil content was estimated. Results show that olive oil and butter are absorbed to a greater extent after 2 hours' stay than mustard oil after 4 hours' stay and the absorption of the latter after 4 hours' stay is greater than cocoa-nut oil after 6 hours stay (Basu and Nath, 1946 *a*)

6. Fats help absorption of Ca and P, and also their utilisation in humans. But cocoa-nut oil causes large excretion of Ca through the intestine (Basu and Nath, 1946 *b*).

7. By Frazer's chylo-micron method it is found that butter, ghee and cocoa-nut oil are rapidly absorbed from human intestine, while ground-nut and sesame oil are absorbed slowly (Nhavi and Patwardhan, 1946)

8. Prolonged starvation lowers blood sugar even to 40 mg per cent and in some cases there is a lowering of glucose tolerance (Bose, De and Mukherjee, 1946).

9. Sectioning of hypothalamus abolishes the hyperglycaemic effect of urethane (De, 1946).

10. Aceto-acetic acid is oxidised *in vitro* by normal blood plasma alone in alkaline medium without H_2O_2 but diabetic plasma is less effective. Amellin accelerates ketolysis *in vitro* of this acid by H_2O_2 in an alkaline medium If amellin is injected simultaneously with aceto-acetic acid in normal rabbits, the latter is ketolysed *in vivo* (Nath and Islam, 1948).

11. Sodium salts of di-acetic acid and β-hydroxy-butyric acid, if injected daily into normal rabbits, cause an initial reduction of blood sugar for 2 weeks or so, followed by a gradual rise of blood-sugar, and ultimately a diabetic state with glycosuria and acetonuria. Ketone bodies may at first stimulate Beta Cells, but later on cause damage to them (Nath and Brahmachari, 1949 *a*).

12. On injection of 100 mg Na-salts of di-acetic or β-hydroxy butyric acids into normal guineapigs, insulin activity of pancreas increases, but if the injection be continued for a long time, there is a gradual reduction of this activity Puryvate is not so active in this respect as the above acids (Nath and Brahmachari, 1949 *b*)

13 Various factors in the production of glycosuria in humans have been discussed (Basu, 1937)

14. Vitamin B_1 causes an acceleration of the inflow of phosphates into and of absorption of glucose from the intestinal loops, but whether the greater absorption of sugar is due to greater inflow of phosphates or independent of it has not been ascertained (Basu and Ray, 1947).

15. Dietetic hepatic lesions and protein deficiency (Rao, 1948)

16. (a) Effects of strychnine, atropine and quinine on the absorption from intestine of glucose, fructose, arabinose and Xylose (Roy and Sen, 1943 *a*). (b) Effect of quinine on the absorption of fat (Roy and Sen, 1943 *b*).

Quinine increases the rate of absorption of all sugars, but strychnine has no effect. Glucose is absorbed more readily from ileum than from duodenum. Quinine decreases the rate of absorption of neutral fats by 23 per cent and fatty acids by 8.5 per cent. This decrease in absorption of fats may be partly due to decrease in rate of hydrolysis of fats by quinine.

17. On evaluating the percentage absorption by balance-sheet method of some normal oils, hydrogenated oils, and thermally treated oils of both varieties, it was found (a) that the degree of unsaturation does not affect absorption, (b) that thermal treatment decreases the absorption of all oils except ground-nut oil, and (c) that the increase in viscosity caused by thermal treatment reduces the emulsification of oils and thereby its absorption (Roy, 1943 and 1944).

18. When a suspension of carotene in glucose solution was introduced into the stomach of vitamin A depleted rats or injected directly into the duodenum after opening the abdomen under anaesthesia and the rats were killed hourly for the estimation of carotene and vitamin A in the washings of duodenum and other parts of intestine and also in liver, a large increase in vitamin A (as determined

by Carr- Price reaction) was found in the intestinal contents, which reached a maximum in about 3 to 4 hours, the amount of carotene decreasing *pari passu* during the same period (Conoor Nut. Res. Lab. Rep. 1948-49).

19. There is no significant statistical difference between Vanaspati (melting pt. 37°C) and ghee in regard to digestibility, and influence on protein, Ca and P metabolism (Conoor Nut. Res. Lab . Rep. 1949-50).

20. By putting persons first on a predominantly vegetarian basal diet, and then on an iso-caloric diet in which animal protein formed 50 per cent of the total protein and then again on the basal diet and estimating the faecal and urinary N_2 during these experiments, it was found that while faecal N_2 varied but little with the changes in the diet, showing that the digestibility of the protein whether of animal or vegetable sources was practically the same, urinary N_2 excretion was distinctly higher on the diet containing a larger proportion of animal protein As the weights of the persons were either steady or showed an increase of not more than 2 lbs. the nitrogen retained in the case of vegetarian diet was not much utilised for the building up of new tissue Further, these results lead to the conclusion that low urinary N_2 in Indians is not due either to low protein intake or to low digestibility of vegetable proteins (Conoor Nut. Res. Lab. Rep. 1949-50).

21. A single intravenous injection of alloxan (300 mg/kg body weight) to 6 monkeys caused the death of 3 of them by hypoglycaemia while 3 others became diabetic without hypoglycaemia being developed On examining the islet tissue of one of the latter, cellular changes were noticed (Banerjee, 1944).

22 After injecting alloxan (200 mg/kg of body-weight) into (a) 3 partially pancreatectomised rabbits and (b) 3 rabbits with intact pancreas and comparing their blood sugar changes with those of (c) 3 other partially pancreatectomised rabbits, not treated with alloxan (all the rabbits having been previously fasted for 12 hours), it was found that rabbits under (a) did not develop hypoglycaemia, but became hyperglycaemic and glycosuric, that rabbits under (b) developed marked hypoglycaemia and died, and those under (c) excreted only traces of sugar and had the usual fasting blood sugar values (Banerjee, 1945).

23. A number of substances were found to prevent the diabetogenic action of alloxan (Banerjee, 1947).

24 Intravenous injection of alloxan (200 mg/kg) into rabbits which were fasted for 7 days and received, during the fasting period daily intra-muscular injection of 100 mg of phlorhizin in olive oil, or into rabbits made diabetic by a previous injection of alloxan does not produce hypoglycaemia. In rabbits fasted and phlorhizinized for 7 days a diabetic type of glucose curve is obtained. These experiments and those under (22) led to the conclusion that alloxan hypoglycaemia is pancreatic in origin (Banerjee and Bhattacharyya, 1948).

25. In alloxan diabetes of rabbits acetonuria sets in early and reaches the

maximum at the end of the 1st week and then gradually decreases to normal levels, this decrease being due to a decrease in ketogenesis and not to increased utilisation of ketone bodies. The partial removal of pancreas does not affect this acetonuria (Banerjee and Bhattacharya, 1949).

26. Rabbits of which the livers were damaged by the previous intake of yellow P or which had persistent glycosuria caused by daily administration of phlorhizin for 7 days, do not develop alloxan hypoglycaemia, but rabbits fasted for 7 days develop this hypoglycaemia within 4 to 6 hours after injection.

In alloxan hypoglycaemia glycogen increases but in insulin hypoglycaemia it decreases in liver.

These facts lead to the conclusion that alloxan hypoglycaemia and insulin hypoglycaemia are not produced in the same way, that the former is extra-pancreatic in origin, due probably to temporary blockage of liver glycogenolysis (Bhattacharyya, 1950).

27. Alloxan and insulin injected rabbits develop convulsions almost at the same time, the degree of hypoglycaemia is similar and there is considerable decrease in the glycogen content of the liver to the same extent. As the symptoms developed in the rabbits injected with alloxan is similar to those of insulin-treated ones it may be suggested that the alloxan hypoglycaemia is pancreatic in origin (Banerjee, Sadhu, Ghose and Chattopadhyay, 1950).

28 Saha and Banerjee (1943) studied the absorption of ferrous and ferric iron from the small intestine of rabbits. Reduction of ferric iron is not necessary before absorption through the intestine. Absorption of iron does not depend on the acidity of the intestinal content.

29. *The specific dynamic action (SDA) nutrients with special reference to the* effects of vitamins and hormones (Sadhu, 1947)

SDA of foods has been measured in a modified Regnault-Reiset apparatus Pyruvic acid lowers SDA of glutamic acid and tyrosine by acting as an amino-acceptor of glutamic acid and tyrosine which are actively transaminated. Pyruvic acid does not lower the SDA of glycine which is not transaminated. Lactic acid behaves similarly to pyruvic acid, as it is easily oxidised in the body to pyruvic acid

Pyridoxine lowers SDA of glucose, glutamic acid and tyrosine, but not of glycine by acting as co-transaminase. Pyridoxine deficiency raises SDA of glutamic acid, but not of glycine.

Vitamin E lowers SDA of glycine and not of glutamic acid. SDA of amino acids is due to deamination in liver which is counter-balanced by simultaneous transamination in which ammonia is accepted by alpha-keto acids, the latter being converted to amino acids. Transamination, as a reversible process, reduces the *irreversible steps of deamination which causes energy loss as heat, and thereby*

reduces SDA of amino acids. This SDA of amino acids depends on the relative degrees of deamination and transamination they undergo in the body.

30. Thiouracil, basal metabolism and specific dynamic action (Sadhu and Brody, 1947).

Rats on thiouracil diet showed a depressed and delayed SDA for glycine and glutamic acid, but not for tyrosine, thus substantiating the theory that tyrosine competes with and tends to displace thyroxine from the active centres of issue cells.

31. Pyridoxine, ketonic acids, and specific dynamic action (Sadhu and Brody, 1947 *c*) SDA of amino acids depends not only on their nature, but also on the presence of pyridoxine, which functions as a prosthetic group in the catalytic system for transamination, and on the presence of alpha-keto acids, such as pyruvic acid, which function as amino acceptors.

32. Physiological mechanism of experimental goitrogenesis (Sadhu, 1948 *a*).

Thiouracil acts as a goitrogen by reacting with iodine, inhibiting peroxidase activity, competing with tyrosine for iodination, and with di-iodotyrosine for oxidation and also competing with uracil in the body.

Aniline derivatives produce goiter partly by competing with tyrosine for available iodine in body and with di-iodotyrosine for the enzyme system necessary for its conversion to thyroxine.

Goitrogens have been classified into three categories with several sub-classes depending on the stages in the biosynthesis of thyroxine. The first class of goitrogens (e g thiocyanate) acts by interfering with the iodide-concentrating mechanism of thyroid as thiocyanate. The second class prevents oxidation of iodine into iodine which reacts with tyrosine to form di-iodotyrosine, either by acting on cytochrome system, (e g. carbon monoxide, azide, sulphides, arsenic etc.) or by inhibiting peroxidase activity (e g thiourea, thiouracil, etc.) or by competing with tyrosine for free iodine (e.g p-amino benzoic acid and aniline derivatives). The third class prevents oxidation of 2-di-iodo-tyrosine molecules to thyroxine by competing with di-iodotyrosine for the enzyme systems which convert this to thyroxine, as for example the aniline derivatives. Many act in more than one step.

32. Correlation between the lactose content of milk and the cerebroside and choline content of brain (Sadhu, 1948 *b*)

Brain cerebrosides show a high posititve correlation with lactose percentage of the milk of the species. There is a slight positive correlation between choline content of brains and the lactose content of mother's milk. It is suggested that sphingosine can reversibly take up galactose or choline phosphoric acid to form cerebrosides or sphingomyelins and thus cerebrosides may function as choline sparers.

Food and Dietetics

1. Nijogi and Patwardhan with others (1939, 1940, 1941 *a*, *b*, 1943) determined the basal metabolism of children and adults and the nutritive values of

Indian vegetable foods and fish liver oils. They also made a survey of Bombay dietary and formulated cheap well-balanced diets.

2. Chitre, Desai and Bharani (1948) assayed the nutritive value of some pure strains of cereals and pulses grown in Bombay state.

3. Subrahmanyam and Banerji (1950) determined the biological value of various fish proteins and found that the hilsa fish protein has the highest biological value.

4. Subrahmanyam and Banerjee (1950) also found that the biological values of fish proteins progressively increase when dried between 60°C and 100°C and also when a factor having anti-trypsin activity is removed by extraction with HCl.

5 Subrahmanyam and Banerjee (1950) further found that the ionisable components in some vegetables increase after digestion in contact with sheep's intestinal mucosa at 37°C for 6 hours

6. Subrahmanyam (1951) determined the biological value (B V) of (a) basal diet deficient in tryptophan and nicotinic acid, (b) basal diet with tryptophan in varying amounts, and (c) basal diet and, tryptophan and nicotinic acid in varying amounts, and found that the addition of nicotinic acid increases the B V , possibly by sparing tryptophan for its role as an essential amino-acid

7. Subrahmanyam (1951) incubated fresh liver slices of rats depleted of B vitamins, with tryptophan and members of the B group of vitamins, viz. thiamin, ribo-flavin, pyridoxin, folic acid, choline and pantothenic acid separately and found that tryptophan is converted into nicotinic acid in the presence of thiamin and pyridoxin, choline increasing the action of pyridoxin.

8 Basu and Basak (1939) found that in a protein-free diet, N_2 excretion in urine is 1.499 g. and in faeces 1.133 g. per day and calculated the total excretion of N_2 per kg body weight to be 0.05 g From the above data they calculated the following (a) minimum protein requirement for maintenance per 70 kg body weight on rice diet (rice, pulse and vegetables) — 37.5 g, (b) on wheat diet (whole wheat, pulse and vegetables) — 34.7 g, (c) and the average protein requirement — 46 4 g, (d) average values for digestibilities of rice and wheat diets are 62 per cent and 78 per cent and the biological values of mixed proteins of these diets are 75 per cent and 66.5 per cent respectively, (e) the retention of protein with iso-caloric values of these diets is greater with wheat than with rice diet, (f) sugar acted as protein sparer.

9. In chillies and coriander seeds cystine is absent, while tryptophane and histidine are present in very small amounts (Narasinhamurthy, 1938).

10. Steam-dried Rohu fish — B V. of proteins @ 5 per cent, 10 per cent and 15 per cent levels of protein intake — 82.3, 78 9 and 72.5 respectively. Steam-dried Hilsa fish — B.V. of proteins @ 5 per cent, 10 per cent and 15 per cent levels

of proteins intake — 78 0, 69.5 and 62.1 respectively. (By balance-sheet method).

Appreciable supplementary relations exist between proteins of Rohu fish and proteins of such pulses as Khesari (*Lathyrus sativus*) and Musur (lentil) for the maintenance of N-equilibrium of the body.

In Rohu the extractable N_2 is 96 4 per cent of the total N_2, but in Hilsa it is 93 0 per cent. Tryptophane content is the same in both but Rohu contains more tyrosine (Basu and De, 1938)

11. Amongst the various foods, nuts, pulses, cereals and fishes contain the largest amount of copper and cow's milk contains a very low amount (Chowdhuri and Basu, 1939).

12. The fat content of Dhain (fish) — 12 per cent, Pangas — 10 8 per cent and Sarputi — 9.48 per cent Pabda contains the highest percentage of protein, viz. 19.2 per cent, Fe present in the body of fish in combination with protein is liberated by digestion and becomes available Peptic digestion is more effective than tryptic digestion. During egg formation available iron of fish muscle decreases and is concentrated in the roe (Saha and Guha, 1940).

13. Availability in percentage of Ca in ragi — 68, Cambu — 89 and Cholam — 84. Availability in percentage of P in ragi — 58, Cambu — 74, Cholam — 67 and rice — 64 (Giri, 1940).

14. Proximate principles of certain foods were estimated (Mitra and Mitra, 1941).

15. Food values of fish — protein — 19 23 per cent, Ca-0 006-0 90 per cent.

P — 0.150-0 350 per cent, Fe — 0 6 mg — 2 5 mg per cent, Cu — 0 01 mg — 0.24 mg per cent (Khorana, Sharma, Rao and Giri, 1943).

16. Tapioca protein contains tyrosine, tryptophane, cystine in fair amounts and arginine in rather large amounts. It is, therefore, of good quality, but its total amount is low Sreeramamurthy, 1945).

17. Bengal gram contains a substance, hesperidin, which has been isolated and is found to be similar to Vitamin P, which is essential for growth of guinea pigs (*Bhagvat, 1946*).

18 Unripe mangoes which are more acid, are richer in vitamin C than ripe mangoes which are richer in carotene and sugar. The increase in carotene and sugar on ripening bears no relationship to the decrease in acidity and vitamin C content. Ripe 'Langras' are richer in both carotene and vitamin C than other mangoes 'Sepia', 'Totafully' and 'Bombai' mangoes are richer in sugar than other mangoes (Basu, Ray and De, 1947 *a*).

19. Vitamin C and carotene content of several herbs and flowers used in Ayurveda have been estimated. 'Neem', 'Vasak', 'Dhania' and 'Babla' thorn leaves are fairly rich in both carotene and vitamin C (Basu, Ray and De, 1947 *b*).

20. Requirements of the normal diet of Bengalees of different age and sex groups have been worked out (Basu, 1946).

21. Cu content of some Indian foods ranges from 0.18 parts per million in milk to 12.5 parts per million in cereals 94 to 96 per cent of Cu added as $CuSO_4$ to wheat or milk is excreted in the faeces. Cu that enters food from vessels is metabolised simarly as added $CuCO_4$ but there is a slightly higher retention and slightly higher excretion in urine.

Rats kept on chapati and milk with 6 to 9 mg Cu as $CuSO_4$ are less active, hairs being less pigmented, and growth rate lower than that of controls (Datta, 1941).

22. Some common deficiency diseases in India (Rao, 1948 and 1950).

23 Effect of choline and methionine on experimentally produced hepatic lesions in rats (Rao, Datta and Krishnan, 1950).

24 Addition of sprouted Bengal gram to the poor North Indian diet causes an increase in weight due to greater development of muscles, and addition of Ca causes increase in weight due to greater development of bones The effects of the two supplements are additive (Pal and Singh, 1938)

25. A very high pulse diet when taken alone or with cereals only causes damage to kidney and liver This can be prevented by taking cod-liver oil, butter, yeast, carrots, green vegetables, radiostol (B D H) etc The preventive substance is a thermo-stable one (Pal and Bose, 1943 *a*).

26 Spices, rich in Ca, P and N_2 added to poor Indian rice diet can offset the ill effects due to deficiencies of these by keeping the Ca and P content of blood normal and the structure of para-thyroids intact (Pal, 1943)

27. L-tyrosine added to Bengalee diet does not either help growth or affect the N-balance, although it is indispensable for the secretion of thyroid and its deficiency leads to histopathological changes (Pal and Bose, 1943 *b*)

28. Cysteine, tyrosine and trytophane in some common edible fishes of Bengal have been determined (Roy and Sen, 1941)

29 Effect of germination, autoclaving and heat processing on the digestibility and biological value of proteins of *Phaseobus aureus roxb* (Sonamung) and *Cicer arietinum* (Cholam) (Sen and Mukherjee, 1949).

30. *Composition of the Cocoanut kernel:* Moisture 2.15 per cent, crude protein 8 27 per cent, ether extractable material 68.41 per cent, ash — 2 10 per cent, carbohydrates (by difference) 19.07 per cent, 58 per cent of the total N_2 is water soluble and consists mainly of proteins and metaproteins. 12 per cent of the total P of cocoanut is phytin P, carotene and riboflavin are almost absent in cocoanut, but choline, pyridoxine, thiamin and niacin are present in small amounts (Conoor Nut. Res Lab Rep. 1946-47)

31. Rats were kept up to 15 months on (a) rice diet (rice 72 parts, milk 7.14,

pulse 3.57, amaranth 7.14, raw plantain 7.14, gingelly oil 1.79, and NaCl — 0 7 parts), (b) on wheat and maize diet (55 parts wheat + 17 parts maize starch replacing 72 parts of rice, (c) wheat diet (72 parts replacing rice) and (d) stock diet — (whole wheat chapati, sprouted gram, milk and mixture of carrots and cabbages) Animals on the stock diet showed no significant change in the chemical composition of liver excepting (a) crude fatty acid which could be as high as 10 per cent particularly with rice diets, inspite of very low fat content of the diet, and (b) cystine and methionine content The ratio of cystine to methionine was 1:4.78 with rice diet, whereas with the stock diet it was 1.0 51. Histological examination showed degenerative changes in livers in animals on rice diet after 15 months. The high cystine content is probably responsible for this liver damage (Conoor Nut. Res. Lab. Rep. 1948-49).

32. Rats kept on poor rice diets (P R D.) were also killed at 12, 15 and 21 months and their livers were examined biochemically The results obtained were

(i) The liver weights in P R D. were comparatively higher than of those on stock diet (S D) at 2 months, but at the end became nearly normal

(ii) The total N-content of livers in P.R D was distinctly lower at 2 months than of those on S D but after 15 months became nearly normal

(iii) After 2 months the crude fatty acid (C.F.A.) content of livers was higher, reaching a maximum of 10 per cent after 4 months, but became nearly normal after 12 months or so

(iv) The methionine content of livers in P R D was throughout lower as it was in the diet also, but the cystine content, contrary to previous finding, did not differ appreciably at any period.

(v) The pathological changes in livers in P.R.D appeared at 15 months and then increased gradually These consisted of fatty degeneration and marked rarefaction (vacuolation) of cytoplasm of liver cells, but no connective tissue hyperplasia.

The change in C F.A. appears to be due to methionine deficiency in the diet, for if to P.R.D. (a) 1 mg choline rat/day (choline exerts lipotropic activity on liver and its deficiency is made good by methionine) or (b) 4 mg DL methionine/rat/day was given, C F A in livers after 4 months, instead of rising to 10.52 per cent, remained at 3.74 per cent or 3.26 per cent which is nearly the same as with stock diet.

The damage to liver is also apparently due to methionine deficiency. That the effects of toxic injuries to liver can be overcome by methionine has been proved by administering (a) CCl_4 to (a) rats on P.R.D., (b) rats on P R.D. + DL-methionine 8 mg/rat/day and (c) rats on stock diet All the rats in (a) died, but all animals in (b) and (c) survived.

33. The phytin phosphorus content of *Phaseolus mungo*, *Phaseolus radiatus*,

Cicer arietinum and *Dolichos lablab* was found to decrease gradually during the process of germination, along with the increase in the phytase activity of the germinating pulse which is maximum either on the third or on the fourth day of germination. The germinated pulse is therefore nutritionally superior to the ungerminated ones (Banerjee and Nandi, 1949).

34. Considerable amounts of calcium, phosphorus and iron are lost with the rice water which is discarded The loss of protein is not appreciable (Banerjee, 1939).

VITAMINS

1 Effects of toxic doses of Vitamin D were noted on the ash, Ca and P content of bones and on the excretion of N_2, Ca and P in albino rats (Patwardhan and Chitre, 1938)

2. Ca content of soft tissues of albino rats in rickets and hypervitaminosis D and the absorption of Ca from intestines were determined (Patwardhan and Chitre, 1940 *a*, *b*).

3 The alleged influence of Vitamin D on the absorption of Ca from the intestine was studied in albino rats (Patwardhan and Chitre, 1942)

4 Ionic products of Ca and phosphate in blood serum of rachitic infants and in experimentally induced Vitamin D deficiency were determined (Patwardhan, Chitre and Sukhatankar, 1944-45).

5 Subrahmanyam and Banerji (1947) found by thiamin clearance test that some people in Orissa excreted only 240 μg Vitamin B_1 instead of the expected amount of 640 μg.

6. Banerjee, Tej and Senapati (1950) showed (a) by perfusing frog's heart, (b) by taking dog's blood-pressure and (c) by recording direct electrogram of frogs heart that a moderate dose of Vitamin B_1 causes initial depression and subsequent acceleration and augmentation of heart-beat without affecting blood-pressure. The depression is less marked after previous atropin administration These effects are not produced with minute doses. In human beings the intravenous injection of a moderate dose of Vitamin B_1 produces severe depression of heart-beat.

7. Biswas and Das (1939) estimated the Vitamin C content of chillies, onion and garlic before and after boiling with water.

8. Carotene when fed as a colloidal solution in water is absorbed by rats, even when the diet contains less than 0.04 per cent fat (Mazumdar, 1939).

9. After a test dose of nicotinic acid, only 12 per cent is excreted (Swaminathan, 1939).

10. Indian gooseberries are a rich source of biologically available vitamin C (Giri, 1939).

11. Guha and Ahmed (1939) showed that Vitamin B_1 excretion of 4 healthy subjects living on different Indian diets varies between 84.0 γ and 228.0 γ, i.e. 5 9 per cent to 19 per cent of the intake, and that the lower intake of B_1 causes reduced output. A large dose of B_1 has a marked diuretic effect.

12. Peripheral nerves of young rabbits, albino rats and fowls, made deficient in vitamin A, were fixed in Muller's fluid and 10 per cent neutral formalin and then stained with Scharlach R. They showed degeneration of myelin sheath to varying degrees (Rao, 1938 *a*).

13. Vitamin A and carotene content of foods is not affected by boiling in open vessels, but loss occurs when butter is melted into ghee by boiling (De and Mazumdar, 1938 *a*)

14. Vitamin A content of liver oil in Rohu fish — 461 I U./g.

„	„	„	„ „	Hilsa	„	— 120 I U./g
„	„	„	body oil in Rohu		„	— 109 I.U./g
„	„	„	„ „	Hilsa	„	— nil

(Basu and De, 1938).

15 Nicotinic acid estimation was based on the yellow color given by pyridine derivatives when acted on by CNBr and aniline (Swaminathan, 1938 and 1941 *a*)

16 Some Indian fish liver oils and vegetables were found to be richer in Vitamin A or carotene than cod-liver oils (De, Majumdar and Sundararajan, 1938).

17. Rats fed on poor Madrasi diet can assimilate carotene present in foods, as is evidenced by increased body-weight and growth rate, such assimilation being uninfluenced by skim milk or calcium lactate (De and Majumdar, 1938 *b*).

18 If Vitamin D is administered orally and subcutaneously to adult albino-rats at a dose of 4500 I U (toxic dose) per day, then a rise in urine N_2, Ca and P and an initial fall in faecal Ca and P, followed by a subsequent rise are noticed. Further, more P is eliminated from the body than Ca and the ash content of the body is reduced (Patwardhan and Chitre, 1938)

19. The niacin content of wheat is greater than that of rice, and of rice greater than that of millets In rice niacin is concentrated in germ and pericarp. When rice is parboiled, naicin diffuses through grains and cannot be removed by milling. Washing and cooking may remove 50 per cent rice niacin Poor rice diet contains less niacin than poor maize diet, which causes pellagra Thus it is difficult to explain why maize is pellagragenic (Aykroyd and Swaminathan, 1940).

20. In balanced diet experiments with rats, if they get 1 mg extra niacin per day in addition to that present in their basal diet, there is no increase in niacin content in their liver, muscle, blood or urine. The addition of niacin to the basal diet containing Vitamin B_1 and riboflavin does not increase their

growth rate. From these it is concluded that rats synthesise niacin in their body (Showrie and Swaminathan, 1940).

21. The tissues (viz. liver, kidney and adrenals) of both normal and scorbutic guineapigs exert the same degree of protective action against the oxidation of vitamin C. This protective factor is present in undialysable and colloidal constituents of tissue extracts (Giri and Showrie, 1940).

22. Rotter's intra-dermal dye-test for vitamin C content of tissues is not applicable to humans (Bakhsh, Kochher and Malik, 1940)

23 Dry skin and phrynoderma respond to vitamin A but not to linolenic acid, and angular stomatitis responds to extracts of yeast but not to niacin (Nicholls and Nimalasuriya, 1940)

24 The vitamin A content of liver and body fats of 16 different species of fish were analysed spectrophotometrically Some of them have in their liver oil 30 times the vitamin A concentration of cod-liver and ¾ of that of halibut liver oil During the sprawning season the vitamin A diminishes but in growth season again increases in liver oil (Seshan, 1940)

25. The vitamin A content of some species of Bengal fish was estimated by biological, tintometric and spectroscopic methods and it was found that the liver oils of many of them contain as much viamin A as cod-liver oil and none of them *showed an absorption band between 345 and 350 Mμ, which is characteristic of* vitamin A_2 (Basu, Rai Sircar and Sen Gupta, 1940)

26 Rabbits fed on vitamin A-deficient diet develop xerophtalmia and lesion in peripheral nerves Ingestion of carotene cures the former but not the latter (Rao, 1940)

27. Vitamin A content of the diet has some relationship to corneal pigmentation (Dhurandhar and Boman Behram, 1940)

28 The vitamin D content of liver oils of Bengal fishes is low (Basu and *Sen Gupta*, 1940).

29. After the intake of vitamin C in heavy doses, a higher percentage of the total excess output of the vitamin is given out in the first 12 hours after the intake than in the following 12 hours, this percentage rising with an increase in the total excess output. Further, the excretion of the vitamin rarely becomes constant and never becomes equal to the amount invested even when the body is completely saturated Under normal conditions and during a period of excess intake of vitamin C, if dehydro-ascorbic acid, combined ascorbic acid, and free ascorbic acid are present in urine, then the ratios of dehydro to free and of combined *to free ascorbic acid lie within a certain range which is almost similar in* both cases, but no apparent relationship exists between dehydro and combined ascorbic acids.

It is further found that no proportionality exists between the average excretion of the vitamin and the state of saturation unless the excretion exceeds 30 mg per day (Basu and Ray, 1940 *a*).

30. Wheat diet causes greater accumulation of niacin in liver, muscle and brain than rice or maize (Swaminathan, 1940 *a*)

31. Scrotal eczema is cured by vitamin A and vitamin B complex without niacin (Karunakaran and Nair, 1940).

32. A method has been described for the estimation of nicotinic acid in blood and other tissue fluids (Kochhar, 1940)

33. Vitamin C augments the contractions of the skeletal muscle induced by single or repetitive stimuli, brings about quicker relaxation and delays the onset of fatigue. Its augmentation effect on the rhythmic contractions of the heart are also well marked but less than on skeletal muscle The slow rhythmic contractions of a strip of intestine are less influenced by vitamin C than cardiac or skeletal muscle An explanation has been suggested for the different response of different types of muscles of the body

After studying the contractibility and fatiguability of the muscle of a finger of several persons before and after they received high doses of vitamin C for a number of days, it was found that they depend upon the condition of saturation of the body with vitamin C (Basu and Biswas, 1940 and Ray, 1940 *b*).

34 A chemical test for the detecttion of vitamin B_6 in foods has been developed (Swaminathan, 1940 *b*)

35 Sodium pyrophosphate protects oxidation of vitamin C by Cu, Fe, Norite, and Cu-albumin complex (Krishnamurthy and Giri, 1941)

36. Raw milled rice loses 60 per cent niacin by washing, whereas parboiled rice loses only 12 per cent (Swaminathan, 1941 *a*)

37. The vitamin B_1 content of millets varies between 3 5 and 4 2 $\mu g/g$, that of wheat does not vary under different manurial conditions, and of rice milled from paddy stored underground does not differ from that of ordinary raw milled rice (Passmore and Sundarajan, 1941).

38 An absorption method for the estimation of niacin has been developed for foodstuffs (Giri and Naganna, 1941 *a*), and also for tissues and blood (Giri and Naganna, 1941 *b*)

39. By a modified Swaminathan method of estimating niacin, it has been shown that most of the niacin of blood is present in the R.B.C (Kochhar, 1941 *a*)

40. Oral administration of niacin brings about the maximum rise in niacin content of blood after about 30 minutes (Kochhar, 1941 *b*).

41. In night-blindness there is a slower rate of visual adaptation and a higher

final visual threshold. Vitamin A (216,000 I.U.) causes improvement in 6 hours and the vision becomes normal after 48 hours (Rajagopal, 1941 *a*).

42. If vitamin B_1 per calorie ratio of the diet falls below 0 25 γ there is a chance of beri-beri developing (Aykroyd and Krishnan, 1941)

43. The normal urinary excretion of Vitamin B_6 in rats as estimated by a colorimetric method evolved by the author, is 400-560 μg (Swaminathan 1941, *c*).

44. The vitamin B_1 content of human milk is 16.3 μg/100 cc. when the infant is under 4 months and 19 5 μg when the infant is over 4 months (Sundararajan, 1941).

45. Spectro photometric and tintometric methods of estimating vitamin A in shark and saw-fish liver oils have been described (Rajgopal, 1941 *b*)

46. A simplified method for the determination of vitamin A status of humans has been described Conjunctival pigmentation or Bitot's spots could not be cured by large doses of vitamin A given for several consecutive days The optimum requirements of vitamin A are found to be 5,000 I U daily and the minimum requirements for boys and adults are 4,000 I.U and 3,000 I U per day respectively (Basu and De, 1941 *b*).

47 A fluorometric method has been developed for the estimation of riboflavin in foodstuffs (Swaminathan, 1942) urine (Swaminathan, 1942 *b*) and urine and tissues of rats (Swaminathan, 1942 *c*)

48 Treatment with lead acetate and preliminary washing with iso-butyl-alcohol gives better results for vitamin B_1 estimation in foods by thiochrome method (Swaminathan, 1942 *d*).

49 Fish, prawn and crabs are good sources of niacin (2 to 4 mg/100 g) (Khorana, Sarma and Giri, 1942).

50 For estimating niacin and niacinamide in biological materials a portion of the 2*N*-HCl extract is heated in the boiling-water-bath for 40 minutes to hydrolyse niacinamide to niacin Protein derivatives and coloring matters are then removed by barium acetate at pH 6 and NaOH at pH 9 5 The excess of Ba is removed as $BaSO_4$. The total niacin is then estimated in suitable aliquots of the treated extract colorimetrically, using CNBr and aniline at pH 7 (Swaminathan, 1942 *e*)

51. Vitamin C is rapidly formed during germination of grains and reaches the maximum after 30-48 hours, after which it remains constant for 3-4 days Green gram develops the highest concentration of vitamin C during germination Dry Bengal gram contains a large amount of vitamin C (Bhagvat, 1942).

52 In tender walnut there is 15 g vitamin C in 8 g fresh material and this is mainly concentrated in the pulp Vitamin C disappears therefrom on ripening (Ranganathan, 1942).

53 15 to 20 per cent of extra-niacin fed to rabbits is excreted in the urine (Swaminathan, 1942).

54. Conjunctival pigmentation with or without xerosis or Bitot's spots is not necessarily a sign of vitamin A deficiency. Vitamin A given parenterally is most effective for treatment of keratomalacia (Kirwan, Sen and Bose, 1943).

55 Hydrolysis of a foodstuff by an enzyme from pig's intestinal mucosa at pH 6·7 liberates and extracts vitamin B_1 and niacin which may then be estimated by thiochrome and CNBr-aniline methods respectively (Bhagvat, 1943)

56. Vitamin C produced no effect on gingival and periodontal diseases of Indian children (Day and Showrie, 1943).

57. Riboflavin deficiency seldom causes ocular complications Angular stomatitis with lesions of tongue and scrotum is due to deficiency of more than one vitamin (Kirwan, Sen and Bose, 1944)

58. A method has been described for the estimation of pyridoxine in foods, based on rate of growth of B_6-deficient rice-moth larvae. (Sarma, 1944).

59. Ragi, rice-polishings, green gram, mustard, cotton seeds and linseed contain a factor which rapidly inactivates vitamin B_1 *in vitro* This factor is non-enzymic in nature (Bhagvat (Sohoni) and Devi, 1944 *a*)

60 Carp muscle and viscera contain an anti-B_1 factor which is enzymic in nature and thermo-labile. They lose their anti-B_1 activity after dialysis for 48-72 hours, the activity being restored by addition of boiled original extract This shows that there are two components of this anti-B_1 factor, one non-dialysable and thermo-labile, and the other dialysable and thermo-stable. This anti-B_1 factor is thus different from the anti-B_1 factor present in ragi, etc (Bhagvat and Devi, 1944 *b*)

61. Vitamin A in shark liver oil is gradually destroyed on keeping, but in livers preserved with salts remain unaffected in potency (Rao, 1944).

62. Ghee has 36 5 I U. vitamin A/gm and carotene 31 per cent of this value A good proportion of these in ghee and fortified margarine is lost during frying with them (De, Ranganathan and Sundararajan, 1946).

63. Natural fats and oils are almost completely (94·99 per cent) utilised by the human body, but hydrogenation of oil lowers its digestibility to 90 per cent (Basu and Nath, 1946)

64. Dark-adaptation test with bio-photometer is a reliable means of detecting low blood levels of vitamin A in humans in the absence of other symptoms of vitamin A deficiency. The low blood level of vitamin A is associated with increased sedimentation rate of blood (Hassan and Khanna, 1947).

65. In Indian carrots β-carotene is 60·80 per cent in red variety and 34 to 52 per cent in orange variety. Xanthophyll is the principal pigment in light yellow, yellow, pink and violet varieties constituting about 75-94 per cent of total carotenoids (Sadana and Ahmed, 1947).

66. If young albino-rats are fed rachitogenic diets, it is found that their high

alkaline serum phosphatase decreases at the end of the 1st week on this diet, rises slightly at the end of the 2nd week, decreases again at the end of the 3rd week and does not increase at all subsequently, although severe rachitic condition ensues, as is evidenced by increased epiphyseal cartilage and decreased bone ash content. It is therefore suggested that in rickets serum alkaline phosphatase may not necessarily originate from bone (Dikshit and Patwardhan, 1947).

67. Vitamin C requirement of Indian adults per day is 1.6 to 2 mg/kg (De and Chakravarty, 1948).

68. Niacin present in rice-diet (7 2-9.6 mg) is sufficient for adult requirement (De and Banerjee, 1948).

69 Niacin has no effect on blood sugar of normal rabbits but raised that of alloxan-diabetic rabbits. It had no effect in experimental diabetes (Banerjee, Ghosh and Bhattacharya, 1948 *a*).

70 *A simple method for the removal of interfering substances in the estimation* of vitamin B_1 in urine has been evolved (Mukundan and Ramasastri, 1948)

71. During germination of legumes, their vitamin C content increases to a maximum between the 3rd and 6th days and then suddenly decreases. Fe, Cu, Mn, or Mannose added to the germinating fluid augments the increase which is maximum with mannose (De and Barai, 1949).

72. During ripening of mangoes, all carotenoid pigments increase, but β-carotene at a greater rate than others An average-sized mango may synthesise 1200 mg of β-carotene in 24 hours (Sadana and Ahmed, 1949 *a*)

73. Carotene from carrots contains an adequate amount of anti-oxidants, so that little protection can be obtained by additional amounts of anti-oxidants at 0.1 per cent level (Sadana and Ahmed, 1949 *b*).

74. Massive administration of niacin does not help the absorption or storage of riboflavin or *vice-versa* (De and Banerjee, 1949).

75 If the protein content of the diet be much below the average requirement, the vitamin storage in liver is seriously interfered with even if there be a liberal supply of fat and vitamin A rich liver oil in the diet (Basu and De, 1941 *a*)

76. If vitamin C (300 to 500 mg) be ingested ½ an hour before the intake of 75 g of dextrose for blood-sugar-curve determination, the rise of percentage of blood sugar is markedly less than that after the ingestion of the same dose of sugar alone. If vitamin C is ingested along with sugar, the rise of blood sugar is at first higher than before, but the level of blood sugar is brought down quickly

77. Vitamin B_1 may be a factor in the production of glucuronic acid in liver (Basu and Ray, 1947).

78 Total vitamin C content of some ordinary foods before and after cooking was determined and it was found that sufficient vitamin C is left in some of them

even after frying and boiling and that vitamin C is not completely destroyed in most of them (Basu and Neogy, 1948).

79. Treatment of Phrynoderma by vitamin A concentrate (Rao, 1938 *b*).

80 By applying Rotter's intra-dermal test for vitamin C nutrition to Vizag people, it was found that hypovitaminosis C is more common amongst poorer children than middle class students. This finding was in consonance with their dietary habits (Reddy and Sastry, 1941 *a*).

81. By applying Rotter's test and ascertaining dietary habits of ophthalmic patients it was found that hypovitaminosis C is associated with ocular inflammatory condition (Reddy and Sastry, 1941 *b*).

82. Applying Rotter's test to a case of scurvy before and after treatment it was confirmed that test-times longer than 10 minutes are indicative of hypovitaminosis-C of varying degrees (Sastri and Reddy, 1942)

83. *A comparative study of the chemical and biological methods of estimation* of vitamin C content of cabbage juice was made (Sengupta, Sarkar and Guha, 1941).

84 Urinary excretion of combined ascorbic acid in pulmonary tuberculosis (Banerjee, Sen and Guha, 1941).

85 Vitamin C content of garden rose hips (Mukherjee and Mukherjee 1947).

86. Inter-relationship of some members of vitamin B complex Riboflavin concentration in liver increases in thiamin-deficient rats. Thiamin concentration in liver increases in riboflavin deficient rats. The concentration of riboflavin increases and of thiamin decreases in the liver of biotin-deficient rats

The activities of aldehyde-oxidase and lactic and succinic dehydrogenases depend on the relative concentration of thiamin, riboflavin, niacin and biotin in tissues (Conoor Nut Res Lab. Rep. 1946-47)

87. (a) Chemical features of riboflavin deficiency have been described.

(b) A gross lack of parallelism between phrynoderma and Xerophthalmia is noticed Phrynoderma has been most successfully treated with linseed oil and yeast extract.

(c) A deficiency disease characterized by a burning sensation in the extremities and associated with symptoms of ariboflavinosis, has been treated successfully by marmite therapy and injection of calcium pantothenate, but was not influenced by thiamin, riboflavin and niacin.

(d) The vitamin A content of shark liver oil is found to vary from 110 I.U. to 37,900 I.U. per g.

(e) The relation between the I.U. of vitamin A per g determined by spectrographic method and Carr-Price value, varies from 43.6 to 64.2 (Conoor Nut Res Lab. Rep. 1946-47).

88. 24-hr. excretion of thiamin of 11 subjects		66 μg to 1200 μg
1-hr. ,,	,, ,, ,,	0 062 μg to 0 12 μg per cc.
24-hr. ,,	riboflavin of 11 subjects	340 μg to 4,401 μg
1-hr. ,,	,, ,, ,,	0.14 μg to 1.4 μg per cc

The day to day variation in a given subject is much less than the variations with different subjects.

Blood pyruvic acid in the case of 20 subjects varied between 0 50 and 1 05 mg, the average being 0 74 mg per 100 cc. No correlation was found between pyruvic acid levels in blood and thiamin excretion in urine in 10 subjects who were examined (Conoor Nut Res Lab Rep. 1948-49)

89. If green gram, cow pea and Bengal gram are germinated under sterile conditions at 30°C in a medium containing tryptophan, the ratio of conversion of tryptophan to nicotinic acid is 70 1 in the case of green gram germinated for 3 days, 89. 1 in the case of cow pea germinated for 4 days, and 113 1 in the case of Bengal gram germinated for 5 days (Conoor Nut Res Lab Rep. 1948-49).

90 By maintaining young rats on (a) pyridoxine deficient, (b) fat deficient, (c) both fat and pyridoxine deficient, and (d) normal rations, it was found that deficiency symptoms (viz, dryness and scurfiness of fur, drying and thinning of hair, scaliness of skin, dermatosis, erythema and swelling of hind paws appeared earliest in (c), then in (b) and last in (a) The I_2 values of liver and kidney fatty acids are lower in both (a) and (b) than in (d) and are still lower in (c) The external symptoms of combined deficiency could be cured by fat alone and only partially ameliorated by pyridoxine (Conoor Nut. Res Lab Rep 1949-50)

91. Urinary excretion of thiamin and riboflavin and blood lactic acid and pyruvic acid and the ratio of these 2 acids were determined in 15 healthy adult male persons kept on (a) on normal diets, (b) diets of known composition, (c) on controlled diets of known compositions for definite periods and (d) after ingestion of 1 mg of thiamin or riboflavin

Results.	*Thiamin*	*Riboflavin*
(a) Intake in mg.	0.8 — 1 3	0.76— 1 91
(b) Excretion in urine in 24 hours (μg)	66—1200	343—4,401
(c) Percentage of intake excreted in urine	8—63 3	40—250
(d) Urinary excretion in 1 hr. during fasting (μg)	3 4 —90	24—143
Average (μg)	15.5	78
(e) Excretion per cc. of urine in 24 hrs. (μg)	0.03— 0 49	0 26— 1.86
(f) Excretion per cc. of urine in fasting in 1 hr (μg)	0 03— 0 39	0.14— 2.00
(g) Test-dose return percentage	3—55	4 4 —85 3

Thiamin. (a) 74 per cent of 98 observations on urinary excretion fell between 100 and 500μg.

(b) There was significant correlation between thiamin per cc of urine in 24 hours' *urine and in 1 hour fasting urine.*

(c) There was no relation between urinary thiamin and its intake or between pre-test-dose level of urinary thiamin and test-dose return.

(d) There was no correlation between thiamine excretion and non-fat calories in the diet.

(e) Blood *pyruvic acid* varied between 0.5 and 1.28 mg/100 cc. Average — 0.9 mg/100 cc.

Lactic acid — 5 95 — 14 88 mg/100 cc

Average — 8.78 mg/100 cc

Pyruvic to lactic acid ratio — 1 9

Riboflavin· (a) Range of riboflavin excretion was 40 to 250 per cent and there was thus no relation to intake, and the test-dose return was independent of daily intake and the level of pre-test dose excretion.

(b) *Riboflavin excretion was influenced by protein and fat content of the diet.* With an increase in protein or fat content, riboflavin excretion decreased.

(c) Sometimes riboflavin excretion was much greater than the intake, suggesting its microbial synthesis in the intestine and its absorption therefrom (Conoor Nut. Res. Lab Rep. 1949-50).

92. Carotene given orally or intra-muscularly to goats does not appear as such in blood, but produces an increase in vitamin A content On intravenous injection of carotene in oil, it disappears from blood within 15 minutes (Conoor Nut Res. Lab. Rep. 1949-50).

93. Rats were rendered rachitic and then given 4000 I U. vitamin D_2 in oil. Their bones on removal and on treatment with calcifying solutions showed 2 to 3 times more extensive calcification than the bones of rachitic animals which did not get vitamin D_2, the width of the epiphysical cartilage being the same in both groups (Conoor Nut. Res. Lab Rep. 1948-49).

94. Clinical evidence shows that severe diarrhoea can be one of the acute manifestations of vitamin A deficiency in children (Conoor Nut. Res. Lab. Rep. 1948-49).

95 Cases of phrynoderma were treated with (a) vitamin A concentrate (b) gingelly oil, (c) gingelly oil and vitamin A concentrate, (d) gingelly oil and marmite, and (e) marmite. Response to treatments (b), (c) and (d) was favourable but vitamin A alone was ineffective. The unsaturation of serum fatty acid was lower in these cases than normal and became higher after 3 or 4 weeks treatment when signs of clinical improvement were also noticed. (vide also 87 *b*). (Conoor Nut. Res. Lab. Rep. 1948-49).

Further clinical investigation and treatment showed that gingelly oil, containing linoleic acid to the extent of 40 per cent of its fatty acid content by itself can cure phrynoderma but marmite, vitamin A, or skim milk individually were without effect. These patients had only 8 4 g fat in their daily food (Conoor Nut. Res. Lab. Rep. 1949-50).

96 Nicotinic acid content of pulses (14 varieties) increases enormously during germination and the maximum value is obtained on the second day of germination (Nandi and Banerjee, 1949 *a*).

97. Both the reduced and the dehydro-ascorbic acid contents of ten varieties of pulses increase considerably as the germination proceeds and the maximum value is obtained on the third day of germination (Nandi and Banerjee, 1949 *b*).

98. Thiamin content of nineteen varieties of pulses is found to increase during germination and the maximum value is reached on the third day of germination. Different varieties of *Cicer arietinum*, (Bengal gram) are found to be the richest source of thiamin both before and after germination (Chattopadhyay, Nandi and Banerjee, 1959 *a*).

99. Riboflavin content of pulses is increased enormously during germination (Nandi and Banerjee, 1950 *a*).

100. A rapid chemical method of estimation of thiamine in biological materials has been described (Nandi, Chattopadhyay and Banerjee, 1949).

101 A fluorometric method of estimation of nicotinamide in food materials has been described (Banerjee and Banerjee, 1950).

102 Trigonelline content of *Phaseolus mungo* diminishes and there is a rise in the nicotinic acid and nicotinuric acid contents during germination Chloretone enhances the production of nicotinic acid in germinating *Phaseolus mungo* to a considerable extent. Similar action is also shown by DL-tryptophane (Banerjee and Banerjee, 1950 *a*)

103 After feeding nicotinic acid to guineapigs there is no increase in the urinary excretion of methyl nicotinamide. Guineapigs do not excrete increased amounts of nicotinic acid when fed tryptophane unlike the rats (Banerjee and Banerjee, 1950 *b*).

104. Effect of germination on the vitamin content of some cereals has been studied (Nandi and Banerjee, 1950 *b*).

105 Deficient utilization of glucose by scorbutic guineapigs is not affected by either hypo or hyperthyroidism (Banerjee and Ghosh, 1949 *b*).

106. Blood sugar and blood acetone bodies are practically unaffected by the injection of nicotinamide in both diabetic and normal subjects (Banerjee and Ghosh, 1949 *a*).

107. A chemical method of estimation of nicotinic acid in urine in the presence of sugar has been described (Banerjee, Ghosh and Bhattacharya, 1948 *b*).

108 Glucose tolerance is significantly lowered, liver glycogen value is significantly diminished and the insulin content of the pancreas is diminished to about one fourth of the normal value in scorbutic guineapigs (Banerjee and Ghosh, 1947).

109. There is significant increase in the size and also in the adrenalin content of the adrenals of scorbutic guineapigs. Demedullated scorbutic guineapigs also show deficient utilization of glucose. This indicates that adrenalin is not responsible for the lowered glucose tolerance in scurvy (Banerjee and Ghosh, 1946).

110. The claim of Wooley that gluco-ascorbic acid is an anti-vitamin of ascorbic acid has been disproved by Banerjee and Elvehjem (1945).

111. Blood chloride values of guineapigs are not altered when they develop scurvy (Benerjee, 1943)

112. The number of islets of Langerhans and the size of the islets are significantly increased in scorbutic guineapigs but the β-cells are degranulated (Banerjee, 1944 *a*, *b*).

113. Intradermal test of Rotter for vitamin C status of the body has been studied by Banerjee and Guha This test is suitable for determination of the vitamin C-nutrition of the body (Banerjee and Guha, 1939, 1940 and 1942; Banerjee, 1944 *c*).

114 Urinary excretion of combined ascorbic acid is not appreciably affected by the administration of a single high dose of ascorbic acid to tuberculous patients. Combined ascorbic acid disappears from the urine of normal human subjects 1-3 days after continued injection or ingestion of ascorbic acid in doses of 70 mg per stone of body weight (Banerjee and Guha, 1941).

115 The stability of ascorbic acid in urine has been investigated in presence of different acids. The presence of combined ascorbic acid in urine has been shown (Banerjee, 1940).

116. One hundred and thirty-eight individuals of different strata of society have been examined with the biophotometer to assess their level of vitamin A nutrition (Roy and Banerjee, 1941).

117. In studies with sixteen diabetic patients it has been observed that the fasting blood sugar value reaches the normal level and the utilization of ingested glucose is improved after administration of 500 mg of ascorbic acid for three weeks Ten of the diabetic patients had not excreted sugar in their urine three weeks after the administration of ascorbic acid (Banerjee and Ghosh, 1950).

118. Stimulating effect of chloretone on ascorbic acid excretion by rats can be largely suppressed if the animals are made deficient in either thiamin or riboflavin. The action of chloretone can be restored by correcting the respective deficiencies by thiamin of riboflavin feeding (Roy and Guha, 1946).

119. Acetylation serves as a useful laboratory technique for detecting cases

of burning feet syndrome due to pantothenic acid deficiency (Sarma, Menon and Venkatachalam, 1949).

120. A yellow coloured compound is excreted by rice-moth larvae when they are fed pyridoxine deficient diets containing tryptophane. The yellow color disappears from the excreta on the inclusion of pyridoxine in the diet. The compound in question may be different from xanthurenic acid (Sarma, 1945)

121. A diet containing a high amount of corn grits inhibited the growth of chicks (Sarma and Elvehjem, 1946).

122. Biological assay methods for pyridoxal, pyridoxamine and pyridoxine have been described (Sarma, Snell and Elvehjem, 1946)

123 Bioassay of vitamin B_6 in natural materials has been described (Sarma, Snell and Elvehjem, 1947).

124 Acetylcholine accelerates onset of fatigue and delays relaxation of skeletal muscle, while thiamin delays onset of fatigue. Thiamin in sufficiently high concentration annuls to various degrees the effects of acetylcholine on skeletal, plain and cardiac muscles It also prevents the inhibitory action of vagus stimulation on heart beats Thiamin reacts with acetylocholine to form acetylthiamin, as proved by experiments with acetates which had effects similar to those of acetylcholine (Sadhu, 1946).

125 Heavy vitamin A feeding reduces basal metabolism and thyroid weight of rats, and also neutralises the increased metabolic effect of thyroxine injection (Sadhu, 1947)

126. Heavy vitamin A administration decreases thyroid weight by lowering the secretion of thyrotropic hormone of the anterior pituitary Potassium iodide slightly lowers thyroid size and depresses thyrotropic secretion or inactivates thyrotropic hormone Iodized vitamin A has no influence on thyroid size or on thyrotropic secretion, confirming the conclusion that vitamin A lowers thyrotropic secretion directly and not by compound formation with thyroxine Thyrotropic hormone was assayed by the response in day-old chicks and confirmed histologically by measuring the height of epithelia in thyroid acini (Sadhu, 1948).

127. Protein-bound iodine was decreased in liver and increased in thyroid after reticulo-endothelial cell block, indicating increased production and storage of thyroxine, though the latter is released in normal amounts (Truscott and Sadhu, 1948)

128. Hypervitaminosis A was accompanied by decrease in protein bound iodine in liver and thyroid and by increase in serum, skeletal muscle and pituitary. This indicates that there is decreased hepatic destruction of thyroxine, with consequent hyperthyroxinaemia; the latter depresses thyrotrophic hormone secretion, producing the observed decrease in both thyroid weight and in thyroid iodine (Sadhu and Truscott, 1948).

NUTRITION

1. Niyogi, Patwardhan and Mordecai (1939) determined the basal metabolism of some healthy Bombay men and women by Sanborn's graphic metabolism tester (bell-type Spirometer) and obtained the following results·

Average O_2 consumption & heat production
for men — 187 cc/min. and 34.5C/sq m./hr.

Average O_2 consumption & heat production
for women — 152 cc/min. and 32 05C/sq m/hr.

The average total calorie requirements for men and women, per day are 2604 and 1875 respectively.

2. Aykroyd and Krishnan (1939 *b*) found that tapioca diet is fatal to young rats, but when supplemented with casein or skim milk powder, it not only keeps them alive but causes an increase in their weight They concluded that tapioca is unsatisfactory as a staple food.

3. Swaminathan (1939) showed that Ca intake in varying levels does not affect the biological value of proteins.

4. Aykroyd and Krishnan (1939 *c*) showed that the addition of calcium lactate to the diet of Indian children increases their height and weight.

5. Basu, Basak and Rai Sircar (1939) found (a) that the maintenance requirements of P and Ca of Indians are 1.001 g and 0 388 g per day per adult of 70 kg body weight, (b) that typical vegetarian rice diets (rice — 600 g, Ca — 0 15 to 0.2 g and Ca: P — 1/6) fail to maintain Ca-Balance and are inferior to typical wheat diets in the respect and (c) both these diets maintain P balance

6. Sokhey and Malandkar (1939) studied the B.M R. of 60 normal Indians by open-circuit method with Tissot-type gasometer and found that it is 8 per cent lower than the Aub-DuBois standard. This is due to low protein intake and not tot any racial or climatic factors

7. Patwardhan and Chitre (1938) administered 3 different diets with different Ca P ratios to rats and found that while the diet influenced the mean values of Ca and P in soft tissues, viz. muscle, brain, spleen, kidney, testes, liver, heart and lung, Ca. P ratio exerts no influence on such values. Thus Ca in lungs is greater than in brain which is again greater than in liver.

8. Stomatitis in children caused by a diet consisting mainly of milled rice, is cured by eggs and yeast autoclaved in alkaline medium, but soya bean has no curative effect (Aykroyd and Krishnan, 1938).

9. Addition of fresh skim milk to the diet of school children enhances their growth and improves their general health (Krishnan, 1938).

10. The iron content of leafy vegetables, condiments and spices, usually considered to be good sources of Fe, have been determined by the α-α'-di-pyridyl method and found to contain Fe of low availability (Ranganathan, 1938).

11. Phytin-P content of 67 Indian foods were determined. It was found that in cereals and pulses a large percentage of total P is phytin-P, whereas in vegetables phytin-P is either absent or small in amount. condiments and spices occupy an intermediate position (Sundararajan, 1938).

12. Rats fed on South Indian diet with a supplement of phosphate show no change in growth, but if Ca be added, there is an increase in growth. In South Indian diet with or without the addition of phosphate, changes indicative of hypofunction of parathyroids take place (Pal and Singh, 1938).

13. In cereals 50-70 per cent of the phosphorus is present as phytin-P and is not available for nutrition. Phytin increases on soaking seeds in water and on ripening of grains Phytase activities of tubers, vegetables and leaves are higher than those of cereals (Giri, 1938).

14. 'Pala' (*Curcuma zeodoria*) is rich in available Fe and can therefore be used to reinforce the milk and cereal foods of children (Goswami and Basu, 1938).

15. Aykroyd, Madhava and Rajagopal (1938) investigated malnutrition of persons by the measurements of A C H index.

16. Proteins of a mixed vegetarian diet are of fairly high biological value for the maintenance of N equilibrium of adult rats, but small amounts of animal proteins (especially of milk) are necessary for satisfactory growth of young rats (Swaminathan, 1938).

17. Green gram, field pea, lentils, khesari and arhar 'dals' contain carbohydrates which are nearly completely available, but the carbohydrates present in soya bean and Bengal gram are largely unavailable The acidity, as determined by analysing the ash, is least in green gram and highest in lentils (Basu, Bose and Quader, 1939).

18. Diet surveys in the Nilgiris and Travancore showed protein deficiency on account of large intake of tapioca (Krishnan, 1939).

19. On examination of the fat content of Bengal fishes it was found that Hilsa contains the largest amount (19.4 per cent) and Koi and Bhangar came next 8 8 per cent) (Saha and Guha, 1939).

20. The maintenance requirements of Mg is 0.429 g/day. This can be satisfied if Indian dietaries are liberally supplemented by vegetables and legumes. Whole wheat diets maintain better Mg balance than rice diets (Basu and Malakar, 1940).

21. *In rickets* there is diminution of Ca in brain and liver and increases in lung, other tissues remaining unchanged *In hypervitaminosis D* there is increase in Ca in muscles, brain, spleen, testes, liver and heart, but no change in lungs and kidneys.

By introducing $CaCl_2$ or Ca-lactate in the duodenal canal of anaesthetised dogs and estimating Ca and P levels in portal blood serum and mesenteric lymph, it was concluded that Ca absorption takes place through both channels (Patwardhan and Chitre, 1940).

22. Growth of rats on food prepared in tinned brass vessels is 15 per cent lower than of controls getting the same food prepared in glass vessels. 90 to 95 per cent of the ingested tin is excreted in faeces and a little only in urine (Datta, 1940).

23. Mottled dental enamels was found in South Indian children on account of the presence of fluorides in drinking water (Raghavachari and Venkataraman, 1940).

If fluorine present in water exceeds 1 part per million then mottled enamel occurs (Pandit, Raghavachari and Subba Rao and Krishnamurthy, 1940).

24. Liberal supply of vitamin C to monkeys prevents to some extent fluorosis caused by fluorine feeding through water (Pandit and Rao, 1940).

25. The B.V. and digestibilities of proteins of mixed rice and mixed wheat diets are 60 and 80 per cent respectively for rice and 59 and 89.5 per cent for wheat. (Basu, Basak and De, 1941).

26. The bearing of nutrition on preventible blindness and eye diseases in Bengal has been discussed (Kirwan, Sen and Biswas, 1941).

27. There is an increase in B.M.R during hot, humid summer, although it is low on the average in Bombay probably because of racial characteristics. (Niyogi, Patwardhan and Sirsat, 1941).

28. By investigating rats at 5 per cent protein intake by the balance sheet method it was found that parching increases the B.V. of proteins of green gram, horse gram, field pea, jowar, ragi and parboiled rice. (Acharya, Niyogi and Patwardhan, 1942).

29. By the method of isolated loop technique it has been found that vitamin D has no influence on calcium absorption. (Patwardhan and Chitre, 1942).

30. If monkeys are fed on poor rice-diets, chronic diarrhoea with atrophic changes in small intestine, especially, of its lower ⅓rd, and with degeneration of intramural nerve-plexus sets in. (Rao, 1942).

31. Supplement of eggs to Bengali diet has a beneficial effect on growth. (Macdonald and Bose, 1942 and 1945).

32. Betel leaves with lime, if chewed properly, render available Ca for utilization in the body, (Basu, Basak and De. 1942).

33. The bones of small fish form a good source of nutritionally available Ca and P. (Basu, De and Basak, 1942).

34. Proteins of goat's milk are found to have an inferior B.V. to those of cow's and buffalo's milk, when tested by rat-growth method. (Mitra and Mittra, 1942 *a*).

35. The casein of ass' milk has more P, and arginine-N_2 than that of other

milks, and the lactalbumin of ass' milk has more lysine but less tryptophane, tyrosine and cystine than that of other milks. (Anantakrishnan and Lahiry, 1942).

36. By balance-sheet method it is found that at 10 per cent protein intake, B.V. of cow's milk proteins is greater than that of buffalo's or goat's milk and that the B.V.of proteins of the latter 2 milks is identical. The digestibility of the proteins of these different milks is the same. (Mitra and Mittra, 1942 *b*).

37. By balance-sheet method it is noted that antuitrin G (Parke Davis) i.e., anterior pituitary extract, causes an increase in Ca retention and a decrease in faecal Ca excretion. (Krishnan, 1942).

38. Young rats, 4 weeks old, were placed on 4 diets : (a) skim milk supplying all Ca, (b) and (c) skim milk was replaced by dried cabbages and lady's fingers respectively to provide the same amount of Ca and (d) half of the skim milk was replaced with dried drumstick to provide the same amount of Ca as in skim milk diet. After 8 weeks the animals were killed and their bodies were analysed for Ca. The calcium utilization $= \frac{\text{Ca retention}}{\text{Ca intake}}$ is 0.87 for skim milk, 0.71 for lady's finger, 0.82 for cabbage and 0 70 for drumstick (Basu and Ghosh, 1943 *a*).

These vegetables were also given to an adult human and it was found that all of them had a favourable effect on Ca balance. Amaranath, rich in oxalate, was also found to be a good source of Ca. (Basu and Ghosh, 1943 *b*).

39. Casein as well as Ca-lactate promote growth of young rats fed a basal South Indian rice diet Butter alone has an adverse effect on growth rate and general condition. Butter and casein convert the negative effect of butter into a positive effect. (Mason, Theophilus and Frimodt-Moller, 1945).

40 The growth rate of rats on various fats and oils is as follows : Cow butter > buffalo butter > ground nut oil > mustard oil > cocoanut oil > sesame oil. (Basu and Nath, 1946).

41. The B.V. of proteins of mixtures of rice and pulse fed in different proportions to 6 humans varies from 54 to 64, but when pulse is substituted by milk, it varies from 60 to 71. (Mitra and Verma, 1947).

42. Soya-bean milk proteins are 90 per cent as efficient as casein for haemopoiesis in rats. If cow's milk curd protein is replaced by an equivalent amount of soya-bean-milk-curd protein and fed with poor South Indian rice diet, mixed proteins of both diets are utilised to about the same extent. (Desikachar, De and Subrahmanyan, 1948).

43. The B.V. of proteins of cereal mixtures in a rice-eater's diet was investigated by feeding trials with humans. (Mitra, Verma and Ahmed, 1948).

44. When soya-bean milk fortified with Ca phosphate was fed to young growing rats, they retained 82 per cent Ca and 87 per cent P. (Karrani, De and Subrahmanyan, 1948).

45. Cocoanut, cotton-seed and sesame cakes are better than ground-nut cakes as a supplement to South Indian diet. (Kuppaswami, Giri and Subrahmanyan, 1949).

46. With soya milk the growth rate of infants is normal and there is no digestive trouble. The utilization of its proteins is 86 per cent of that of cow's milk proteins and no difference in utilization of Ca and P of two milks is noted. (Desikachar and Subrahmanyan, 1949).

47. Lucerne powder fed at 10 per cent level is a good supplement to South Indian diet. (Subrahmanyan and Sur, 1949).

48. The physiological availability of thiamin, riboflavin and niacin present in cereals and pulses shows that the availability of thiamin is higher than that of the other 2 vitamins. The availability of these vitamins present in cooked cereals and pulses, when compared with that of the fresh foods, is found to be much less. (Chitre *et al*, I.C.M.R. Rep. 1949-50).

49. On curdling of milk, its niacin content decreases to nearly ½ its value after 72 hours, thiamin increases to a slight extent, and riboflavin increases appreciably. (Chitre *et al*, I C.M.R. Rep. 1949-50).

50. The total cholesterol content in liver and brain, the total fatty acid content in liver, and the phospho-lipid content of brain increase when rats are fed on hydrogenated ground-nut oil with or without α-tocopherol, but I_2 value of the fatty acids of tissues shows no significant difference The phospho-lipid content of muscles of rats increases on feeding with ground-nut oil and α-tocopherol, but decreases with ground-nut oil. (Chitre *et al.*, I C.M R. Rep. 1949-50).

51. Basal metabolism was determined on 43 days on the same subject during different seasons in the course of 14 months. The results were strikingly uniform, being within 2 per cent of the average. The B M.R during summer is slightly higher than that during winter. (Rahman, 1939)

52. When guineapigs (male or female, young or old) are given diets containing 'khesari dal' at a level higher than 50 per cent, they do not grow, but develop extreme reluctance to move about, alopecia, dermatitis and in certain cases trophic ulcers in hind legs but not paralysis. Feeding khesari gram at 30 per cent level with 50 per cent wheat flour does not produce these symptoms These symptoms cannot be alleviated by vitamins A, C and B-complex. (Conoor Nut. Res. Lab. Rep. 1946-47).

53. On tapioca diets rats lost weight and 4 out of 6 died. When 30 per cent of tapioca was replaced by cocoanut-cake, they grew at the rate of 8 g per week.

Food yeast given in the ratio of 0.5 oz. to 23 parts of poor rice diets gives the best growth in rats in comparison to that in the higher levels of intake of the basal diet. The same growth is attained if yeast is replaced by ground-nuts — 0.5 to

1.5 oz., red gram and black gram — 1.5 to 3 oz., but 1.5 oz. of skim milk powder gives a much better growth. (Conoor Nut. Res. Lab. Rep. 1946-47).

54. *Growth experiments with rats:* (12 in each group) . I. Age — 4 weeks, weight — 40 to 50 g. given a basal diet containing starch — 53 per cent, skim milk powder — 30 per cent, dried brewer's yeast — 5 per cent, salt mixture — 4 per cent bi-weekly doses of 110 i.u. Vitamin A and 70 i.u of vitamin D per rat per week and fat (which is ghee or refined ground-nut oil, or vanaspati with 19 per cent iso-oleic acid, or vanaspati with 9 per cent iso-oleic acid, or refined cocoanut oil in different experiments) and conducted over 8 weeks showed no significant difference, but the following peculiar features were noted :

(a) In ghee and cocoanut oil 90 per cent matings were successful and in others only 50 per cent were successful.

(b) Gestation period — 25 days with ghee, 32 days with cocoanut oil and vanaspati and 47 days with refined ground-nut oil.

(c) The tendency of the mother to eat the young — highest with refined ground-nut oil, absent with ghee and intermediate with others.

(d) Average no. of living young ones on the 30th day, per litter — ghee — 5.5, refined cocoanut oil — 3.2; vanaspati — 1 3 and 1 1; basal diet without fat — 0.3; refined ground-nut oil — nil

(e) Haemorrhages at the angles of the eyes before the weaning time — ghee — 12 per cent, cocoanut oil — 25 per cent, vanaspati with 19 per cent iso-oleic acid — 100 per cent, the same with 9 per cent iso-oleic acid — 60 per cent, basal diet without fat — 100 per cent.

(f) Opacity of the cornea developed at a late stage, particularly with fat free diet.

II. No appreciable difference in the nutritive value of milk and curds was detected except a significant decrease in niacin content of curds.

III. Experimental lathyrism could be produced with khesari gram in dogs but not in monkeys.

IV. Lipotropic activity of pulses has been investigated. (Conoor Nut. Res Lab. Rep 1947-48).

55 By keeping 4 pups on a diet containing khesari gram at the 91 per cent, level one animal showed stiffness of hind legs and in another signs of hind leg parosis became visible. These symptoms were mild and did not progress in spite of continuous feeding with lathyrus diet for a year, but rather became milder Growth with this diet compares favourably with the controls, and blood examination did not reveal any difference. No toxic effect could be induced in guineapigs with this diet. (Conoor Nut. Res. Lab Rep. 1948-49 and 1949-50).

56 Rats kept on a synthetic diet in which (a) duck egg white (D E.W.) or

(b) hen egg-white (H.E W.) was the sole source of protein, showed at 8 4 per cent protein intake, growths of 21 g. and 65 g. respectively in 8 weeks, but at 25 per cent protein level the differences in growth were considerably narrowed. It has been shown that histidine content of D.E.W. is nearly half of that of H.E.W. and that the digestibility of the former is lower than that of the latter. (Conoor Nut. Res. Lab. Rep 1949-50).

57. Cooked diets of thirteen different hostels in Calcutta were analysed for ether-extract, protein, calcium, total phosphorus, phytin phosphorus, total iron, ionisable iron and copper in three distinct seasons, namely, summer, monsoon and winter. The diets consumed in monsoon were more deficient in every respect than the diets in April and December, which themselves were partly deficient. It was found that the figures obtained by a survey of the raw food stuffs consumed were misleading Actual analysis of the cooked diet should be preferred (Banerjee, 1941).

58. A diet survey of families with leprosy was undertaken (Aykroyd and Krishnan, 1939 *a*).

59. Mitra (1941) studied the dietary and physique of mining population in Jharia coal fields, Bihar.

60. Bhave (1941) carried out diet surveys in the Central Provinces and Berar.

61. Mitra (1942) carried out an observation on the diet and nutritional status of an aboriginal tribe of Bihar.

62 Bhave and Bopaiya (1942) undertook a diet survey and investigated the haemoglobin levels of the inhabitants of Coorg

63. A diet survey of Nazimabad district in Nizam's Dominion was carried out by Daver and Ahmad (1942).

64 Lactose has been shown to be related to brain size and enters into the composition of cerebrosides of nerve tissues It may replace the choline-phosphoric acid radical of sphingomyelin and thus act as choline-sparer. (Sadhu, 1947).

BIOCHEMISTRY

1. Patwardhan and Chitre (1938) analysed the Ca and P content of soft tissues of normal rats.

2. Antibody globulins (i e. γ-globulins) have less affinity for H^+ ion than normal serum globulin, as is shown by titration with sodium hydroxide. (Biswas, 1938).

3. Adrenaline oxidation by phenolase is inhibited by HCN, vitamin C, or ephedrine. (David, Krishnaswamy and Srinivasan, 1940).

4. Peptone preparation for bacteriological work has been made using papain. (Krishnan, and Narayanan, 1941).

5. For the estimation of ammonia — N_2 in biological materials, distillation over magnesia is not suitable in the presence of protein, but distillation in presence of phosphate buffer of *p*H 7 4 gives more accurate results. (Sen, 1942).

6. The estimation of the precursor of nicotinic acid in cereals and pulses has been made by modified method. (Chitre *et al*, I C M.R. Rep. 1949-50).

7 In the ejaculated semen of buffaloes, sheep and goats the total amount of reducing substances as well as of fructose and ascorbic acid vary widely from week to week. Fructose constitutes 70 per cent of reducing substances in buffalo semen and 85 per cent in the semen of sheep and goat, but is absent in fowl semen. Ram semen contains more ascorbic acid than buffalo semen. (Roy, Karnik, Luktuke, Bhattacharya and Bhattacharya, 1950)

8. Fructolysis in semen is proportional to sperm concentration. Accordingly with the increase in sperm, there is decrease of fructose and also of methyleneblue reduction time (Roy, Luktuke, Bhattacharya and Bhattacharya, 1950)

Thyroprotein feeding during summer months increases the sperm concentration per ml. on account of the decrease in the volume of semen. It does not affect the total number of sperms, and increases consequently the rate of fructolysis and thereby augments the motility of sperm cells. Abnormal sperms also increase in number (Mukherjee, Roy and Bhattacharya, 1953).

9. Insulin appears to be the most resistant to tryptic attack when other proteins are present in the reaction mixture Proteins which are easily digested by trypsin seem to protect insulin from enzymic proteolysis (Iyenger, 1941)

10. Alkaline phosphatase is present in erythrocytes of sheep, rat, pig, guineapig and man (Ranganathan and Patwardhan, 1949).

11. Sera of human beings, dogs and monkeys have little or no esterase activity but moderate complement activity. (Roy, 1942).

12 Trypsin kinase from blood and enterokinase have been studied (Iyengar, 1942).

13. Serum phosphatase in pulmonary tuberculosis is generally higher than that of normals Oral administration of large doses of natural vitamin C lowers the serum phosphatase of pulmonary tuberculous patient (Rudra and Roy, 1942)

14 In germinating soya beans the ascorbic acid activity is developed to a maximum after 48 hours. (Rangnekar, De and Subrahmanyan, 1948).

15. Production of citric acid and oxalic acid from molasses by fermentation with the help of a locally isolated mucor, has been studied. (Das Gupta, Saha and Guha, 1940).

16. Theoretical and technical aspects of gluconic acid formation have been worked out. (Bose, 1947 *a*).

17. Mechanism of gluconic acid formation by the enzyme system of aspergillus niger *has been investigated.* (Bose, 1947 *b*).

18. Beneficial effect of plant extracts on the yield of penicillin by a number of strains of penicillium notatum and citrinum from a synthetic Czapek-Dox medium has been observed (Nandi, 1943).

19. Aspergillus which produces large amounts of acid does not produce any antibiotic. (Ramchandra Rao and Iya, 1945).

20. *The antibiotic, polyporin, effective against Gram-positive and Gram-negative* organisms has been described. (Bose, 1946 and 1947).

21. Bindal and Sreenivasaya (1944) carried out extensive investigations on the production of fungal enzymes Wheat bran was found to be the most suitable solid medium for the production of enzymes by *Aspergillus oryzae.* Rice bran fortified with 10 per cent ground-nut cake could be used as a substitute for wheat bran.

22. The alpha-amylase fraction from the culture filtrates of *Aspergillus oryzae* has been separated, isolated and crystallised. The crystalline product is mostly hexagonal in shape and showed amylase activity of the order of 1 · 20,000. (Roy, 1942).

23. Isolation of choline esterase from cobra venom and studies on its properties. (Chaudhuri, 1946).

24. Effect of different substances on the activity of cobra venom haemolysin. *Reversible inactivation of haemolysin. (De, 1940).*

25. Crystalline haemolysin has been isolated from cobra venom (De, 1941).

26. Antigenic properties of crystalline haemolysin have been studied. (De, *1942*).

27. *p*H and heat stability, isoelectric point and molecular weight of crystallised haemolysin have been determined. (De, 1944).

28. *Chemical composition of crystalline haemolysin has been investigated.* (De, 1945).

29. Some amino-acids in cobra neurotoxin have been estimated. (Ghosh and Chaudhuri, 1943).

30 Effect of snake venoms on the oxidation of glucose and its metabolites in the cell suspension has been studied.

31. Effect of snake venoms on the cytochrome-cytochrome-oxidase system has been investigated. (Ghosh, Chatterjee and Sinha, 1948).

32. Proteins in rattle snake venom have been described. (Ghosh and De, 1939).

33. Dipeptidase, polypeptidase, carboxypolypeptidase and esterase have been detected in different snake venoms. (Ghosh, Dutt and Chowdhury, 1939).

34. Various reducing agents destroy the neurotoxin of cobra and daboia venoms. (Ghosh, De and Chaudhuri, 1938).

35. Reaction between *Vipera russellii* venoms and its antivenine has been studied. (Ghosh and Kundu, 1940).

36. Investigation on the isolation of the active principles from the venoms of *Bungarus fasciatus* and *Vipera russelli* have been carried out. (Ghosh, De and Bhattacharya, 1939).

37. Effect of cobra venom and its constituents on the synthesis of acetylcholine by the brain cells of rats and pigeons has been studied (Ghosh, De and Sarkar, 1944).

38. Cardiotoxin has been isolated from cobra venom and its molecular weight has been determined. (Sarkar, 1947 *a*, *b*).

39. Effect of different adsorbents on the toxicity of cobra venom has been studied. (Sarkar and Maitra, 1946).

40 Effect of temperature on the stability of cobra venom cardiotoxin has been studied. (Sarkar, Maitra and Roy, 1946).

41. Only a part of the tyrosine in a protein can be transformed to thyroxine *in vitro*, the main part being converted to di-iodo-tyrosine Tyrosine residues able to condense to form thyroxine occupy certain positions in proteins which make them particularly reactive. Hence the ability of a protein to produce thyroxine depends not only on tyrosine content, but also on its structure. (Sadhu, 1949 *a*).

42 There is decreased excretion of radio-sulphur as organic sulphate and increased excretion in total sulphur of urine of rats fed bromobenzene and radioactive methionine, although there is increased radio-sulphur in liver and kidney proteins and decreased count in proteins of blood plasma, muscle and intestinal mucosa Incorporation of methionine in tissue proteins is retarded when there is extra demand for its conversion into cystine. (Sadhu, 1949 *b*).

43. Methionine with S^{35} was fed to a group of rats, some of which were fed diet containing 2 per cent cystine. There is increased excretion of radio-S in the inorganic sulphate and total sulphur of the urine of cystine-fed rats and there is decreased count in tissue proteins except kidney. In the presence of exogenous cystine in diet, very little methionine enters into tissue proteins. (Sadhu, 1950).

HORMONES AND ENDOCRINE ORGANS

1. Nath and Sengupta (1939) showed that on injection of olive oil solution of artosterone for 21 days to sexually immature male rats, there was increase in weight of prostate and seminal vesicle. Thus artosterone is highly androgenic.

2. Sohoni (neé Bhagavat, 1938) has developed an enzymic method for the determination of adrenaline.

3. Anterior pituitary extracts injected into rabbits (1 — 1.5 kg.) cause fatty

infiltration of liver, which cannot be prevented by choline chloride (500 mg/kg) fed for 8 days. (Mukherjee and Guha, 1938).

4. If rats are kept on a poor rice diet, parathyroids are hypertrophied, and there is an increase in peri-vascular connective tissues. (Rao, 1941).

5. It has been noticed that lactation in some animals may be induced by implantation of stilbestrol. The milk yield in such cases is augmented by the feeding of thyroprotein. But in the case of those animals in which lactation could not be induced by stilbestrol implantation, thyroprotein was of no help. (Roy, Bhattacharya, Luktuke and Bhattacharya, 1950).

6. Anterior pituitary extract (A.P.E) induces growth in thyroidectomised, adrenalactomised, thyro-adrenalectomised and di-ethyl-stilbestrol (D S I.) — implanted rats, even when the food intake of all these rats was maintained at the same level as that of the control animals

(a) A P.E. + thyroxin cannot induce more growth that A.P.E. alone in thyroidectomised or normal rats.

(b) A.P.E + adreno-cortical extract (A C E) cannot induce greater growth than A P E. alone in thyroidectomised or normal rats.

(c) Adrenalectomised rats maintained with 1 per cent Nacl solution grow as well as normal rats.

(d) Thyroxine has no influence on growth of normal rats.

(e) A C.E. alone or A C E. + thyroxine inhibit the growth of normal rats. A.P.E. + A.C.E. + thyroxine produce the same growth as A.P.E. alone. (Roy, 1942).

7. The ascorbic acid content of the ovaries obtained from buffalo cows at various stages of the oestrous cycle has been estimated. As between the two ovaries of the same animal, the one in which ovulation had just occurred was found to contain significantly more ascorbic acid. The persistent corpus luteum is characterised by a very low ascorbic acid content. (Roy, Luktuke and Bhattacharya, 1950).

8. When pregnant mare serum gonadotrophin was injected into goats during anoestrus season or pregnant mare serum (P.M S.) used for breeding anoestrus goat, it was found that the threshold dose for follicular growth is about 600 i u., but generalised follicular growth indicated by an increase in the mean follicular diameter is not obtained with doses below 100 i u. Superovulation was frequent with higher doses but no superfoestation was seen. Only 60 per cent of the shed eggs could be recovered from the fallopian tubes and 22.8 per cent of the injected animals which were mated gave birth to young. (Folley, Roy and Greenbouin, 1948).

Excretory Organs

1. Kidney in cases of slow starvation may have only slight nephritis, as is proved

(a) by clinical and post-mortem examination and also by the biochemical examination of blood and urine for urea, N.P.N., uric acid, blood creatinine, the total quantity of urine and the protein in urine, (b) by microscopic examination of urinary sediments and (c) by blood urea clearance test. (Chakravarti, 1944).

2. Subramanyam and Banerjee (1948) found that the urine of randomly selected Ooriyas contains very low total N_2 and urea N_2, low uric acid and phosphates, but normal amounts of creatinine N_2.

3. The chemical composition of urine of Bengalees differs from that of Europeans in having less total N, urea, inorganic sulphate and Ca and more chloride. (Ray and Ganguly, 1938)

4. The average chemical composition of the sweat of Indians is found to be as follows:

Reaction — acid, *p*H — 5.05, Cl' — 0.387 per cent (0.073 — 0.805 per cent), ammonia N_2 — 10.9 — 34.2 mg per cent, urea N_2 — 10.4 — 61.5 mg. per cent. (Chopra, Roy and Biswas, 1940).

5. In cases of stomatitis of dietetic origin, caused by deficient vitamin B complex intake, urinary porphyrin excretion was within normal limits. (Passmore, Sommervile and Swaminathan, 1940).

6. The maximum and standard urea clearance of Indians are found to be 44 cc. and 33.8 cc. which are definitely lower than those of Europeans. (Gokhale, 1941).

7. The amount of N_2 excreted in urine per 24 hours is higher with intake of animal proteins than on that of vegetable proteins, the faecal N_2 being the same with both. The digestibility is the same. Thus the nitrogen retention is higher with vegetable proteins. The low urine N_2 of Indians cannot always be ascribed to low protein intake. (Patwardhan, Mukundan and Ramasastri and Tulpule, 1949).

8. Effect of methyl-ester of the fatty acid from Bouchi oil on skin depigmentation. (Das, 1949).

9. In Hyderabad — Deccan the average maximum urea clearance of 22 healthy subjects varied between 75 and 70 per cent of American standards. (Rahman and Abhyankar, 1945).

Nerve and Muscle Physiology

1. The influence of temperature variation on activities of the small intestine and the optimum temperature for the maximum actions of acetylcholine and adrenaline have been determined. (Chakravorti, 1948).

2. Adrenaline stimulates rabbit's gut in small doses but inhibits in large doses under certain conditions. (Chakravarti, 1949).

3. Rheobase and chronaxie were determined in vitamin A and vitamin B_1 deficient animals and in normal and underfed persons. B_1 deficient animals have

a higher chronaxie than normal animals, although the rheobase is not significantly altered. Ill-fed persons have generally a higher rheobase and lower chronaxie. (Basu, Roy and De, 1947).

4. By stimulating through attached vagus or through acetylcholine isolated strips of dog's stomach through attached vagus and through acetylcholine with the peri-arterial sympathetic remaining attached to the strip, the following results were reported :

(a) Excitation through vagus (the optimum temperature for its stimulation being 30°C) has an inhibitory component.

(b) Tonus is antagonistic to vagus stimulation,

(c) The optimum conc. of Ca — 0 5 to 0.07 *M*, of K — 0.06 *M*, *p*H 7 8 to 7.4;

(d) Vagus or acetylcholine stimulation does not resemble K-stimulation but is like that produced by alternating current. (Narayana and Singh, 1944).

5. The action of adrenaline on rabbit's small intestine is potentiated by ephedrine (Gupta, 1943)

6. Investigations were made regarding (a) the factors which affect Na, K and total base content of the anterior retractor of the Byssus of Mytilus edulis (Singh, 1938 *a*), (b) the effect of the interaction of ions, drugs, and electrical stimulation, as indicated by contraction of this anterior retractor (Singh, 1938 *b*), (c) the isotonic extension and shortening of this retractor (Singh, 1938 *c*), (d) the effect of adaptation to electrical and chemical stimulation on the excitability of this retractor (Singh, 1938 *a*), (e) properties of tonic contractions of this retractor, caused by electrical stimulation (Singh, 1938 *e*), (f) the effect of Ca and some other factors on the excitability of this retractor (Singh, 1938 *f*), and (g) the effect of stretching and of stimulation on the weight, total base and Na concentration of this retractor (Singh, 1939 *a*).

7. Investigations were also made regarding (a) the comparative effects of interaction of ions, drugs and electrical stimulation as indicated by contraction and *inhibition of unstriated muscle (Singh, 1939 b, 1940 a; 1942 b, Singh, Singh* and Muthana, 1947, Khan and Singh, 1947), (b) the effect of temperature on the mechanical response, viscosity and O_2 consumption of this muscle (Rao and Singh, 1940), (c) the visco-elastic properties of unstriated (Singh, 1942 *a*, 1943 *e*, 1944 *d*) muscle, (d) the effect of H + ions and tonic contraction on the viscosity of this muscle (Singh, 1943 *a*), (e) isotonic extension of this muscle (Singh, 1943 *b*), (f) surface and interior effects in this muscle, caused by various ions (Singh, 1943 *c*), (g) reversal effects (Singh, 1943 *d*), (h) the electrical resistance of this muscle and other tissues and the relation of the same to permeability and excitability (Singh and Singh, 1943), (i) adaptation or accommodation in this muscle (Singh, 1944 *a*), (j) the effect of temperature and ions on the impedance *of unstriated muscle and its relation to permeability and excitability (Singh and*

Singh, 1944), (k) excitation in this muscle (Singh, 1944 *b*), (l) its swelling by certain ions and its relation to permeability, excitability, absorption and secretion (Singh, 1944 *c*), (m) the effect of ammonium on its K content and its relation to the contraction produced on withdrawal of chemical substances from around the muscle (Gokhale and Singh, 1945) (n) reversal of the effect of acetyl-choline on frog heart by normal vagus action, tetanisation of the frog heart, graded responses in the frog heart and the effect of electrolyte-free medium. (Singh, Sehra and Singh, 1945 *a*), (o) the mode of action of K on unstriated muscle (Singh, 1945 *a*), (p) the mode of action of drugs on this muscle and the nature of inhibition (Singh, 1945 *b*), (q) the relation of permeability to adaptation in unstriated muscle (Singh, 1946 *a*), (r) comparative physiology of unstriated, cardiac and striated muscle (Singh, 1946 *b*), (s) the effect of some metals, vitamins, anaesthetics and other substances on unstriated muscle (Singh and Singh, 1946 *a*), (t) tonus in unstriated muscle (Singh and Singh, 1946 *b*), (1948 *a* and 1950 *a*), (u) the interaction between ions, drugs and electrical stimulation as indicated by the contraction of avian unstriated muscle and human unstriated muscle and the active elongation of unstriated muscle (Singh, 1949), (v) the mode of action of nerves and the action of direct current on this muscle (Singh and Singh, 1947 *a*, *b*), (w) neuro-muscular transmission in frog's unstriated muscle (Singh and Singh, 1948 *c*), (x) the effect of asphyxia on the mechanical response of this muscle and its relation to tonus (Singh and Singh, 1948 *b*), (y) the tonus and inhibition of unstriated muscle (Singh and Singh, 1949 *c*), and (z) the action of ions and drugs on the effects of asphyxia on the mechanical response of this muscle (Singh and Singh, 1949 *a*)

8. Investigations were also made by the same author on (a) the action of ions and drugs on the effects of asphyxia on the mechanical response of unstriated *muscle (Singh and Singh, 1949 c)*, (b) *Reactions of fatigued unstriated muscle* and their relation to those of cardiac muscle (Singh and Singh, 1949 *b*), (c) Interrelation between various metabolic mechanisms in unstriated muscle (Singh and Singh, 1947 *d*, 1949 *d*, *f*), (d) The action of various substances on the contractile mechanism of unstriated muscle (Singh and Singh, 1947 *c*), (e) Active elongation of unstriated muscle during inhibition, accommodation and relaxation (Singh and Singh, 1949 *g*), (f) Excitation and accommodation in unstriated muscle and excitation at the anode and cathode (Singh, 1943 *f*), (g) the contraction of unstriated muscle produced by change of tension (Singh, 1943 *g*), (h) Similarities between the excitation phenomena in the unstriated muscle and in the retina (Singh, 1944 *e*), (i) An electrolyte-free medium for unstriated muscle (Singh, 1944 *f*), (j) Tonus in striated muscle (Singh and Singh, 1946 *c*), (k) sleep as an adaptation phenomenon (Singh and Singh, 1946 *d*), (l) Tonic and active elongation of unstriated muscle (Singh, 1944 *g*; Singh and Singh, 1948 *d*), (m) Acetylcholine transmission and electric transmission at nerve-

endings (Singh and Sehra, 1945; Singh and Singh, 1945), (n) An electrolyte-free medium for the frog heart and graded responses of the heart muscle (Singh, Sehra and Singh, 1945 *b*), (o) K-stimulation and acetylcholine and the mode of action of the latter on unstriated muscle (Muthana and Singh, 1946; Singh and Singh, 1947 *c*), (p) Racial characteristics of unstriated muscle (Singh, Singh and Muthana, 1946), (q) Tetanisation of the heart (Singh, Sehra and Singh, 1944), (r) Active relaxation of unstriated muscle during inhibition produced by adrenaline (Singh and Singh, 1950 *c*, *d*), (s) Absorption of O_2 from the subcutaneous tissues and from the peritoneal cavity and stomach (Singh, 1932-33, 1934-35), (t) Intravenous injection of O_2 (Singh, 1940 *b*), and (u) Relation between experiments on isolated muscle and isolated strips of myosin (Singh, 1943 *b*).

NERVOUS SYSTEM AND SENSE-ORGANS AND TISSUE CULTURE

1. *Degenerative changes in distal segments of nerves following transection are* clearly brought out when frozen sections, cut longitudinally and mounted in glycerine, are observed in polarized light between two crossed nicols. (Rao, 1938).

2 Burridge's theory of colour vision and Naidu's experimental support of this theory have been shown to be untenable. (Basu, 1946).

3. Baneful effects of noise on human beings have been discussed. (Basu, 1939).

4 Afferent neurones in the small intestine, gall bladder and pancreas have been traced and their segmental distribution determined It was shown that the afferent impulses for the pupillo-dilator and other reflexes travel from the above sites through both the right and left sympathetic chains and enter the spinal cord through several dorsal roots; those from the small intestine through the 8th thoracic to the 1st lumbar and from the gall-bladder and the pancreas through the 6th thoracic to the 1st lumbar roots. (Sinha, 1947-49).

5. Thermal changes and brain potential (Das, 1946).

6. Effect of certain growth-promoting hormones on tissue explants. (Chopra, Das and Mukherjee, 1940).

7. Growth of fibroblasts in tissue culture under the action of vitamin. (Das, 1949).

8. The effects of cardiac drugs on heart tissue explanted *in vitro*. (Das, 1947).

MISCELLANEOUS

1. Incidence of several diseases in cases of slow starvation and their treatment suggested from hospital experience. (Aich, Chakravorty and Chandra, 1944).

2. Biochemical and post-mortem histo-pathological studies of 407 cases of slow and prolonged starvation were made. The blood sugar levels were found to be very low in cases of slow starvation, but there was no symptom of hypoglycemia. There were considerable changes in intestine and pancreas. (Chakravarty, 1948).

.. 3. Das, Banerjee and Subrahmanyan (1949) noticed diminution of total proteins, reversal of albumin-globulin ratio and marked diminution of lipide-P in blood in cases of liver cirrhosis in Cuttack Medical College Hospital.

4 Antibodies in anti-diptheric horse-serum are absorbed less rapidly after intra-muscular injection in rabbits, when they are mixed with gelatin, or gum-acacia, or milk-fat which increase the viscosity of the serum (Lahiri, 1939).

5. In ten cases of heat stroke the following chemical changes in blood were noted :

(a) Retention of nitrogenous constituents

(b) Increase in creatinine and phosphate

(c) Low calcium

(d) Low chloride in blood and urine

(e) Increase in lactic acid. Presence of albumin and casts etc. in urine suggested kidney insufficiency (Chakravarti and Tyagi, 1938).

6. Lead is present in almost all tissues of the body but its concentration is high in bones, teeth and hair. Skin contains a low amount. Ovary has none, while testes contains it. Tissues of Europeans have a higher content than those of Indians. (Bagchi, Ganguli and Sirdar, 1939).

7. If the liver of dogs is damaged by the oral administration of ccl_4 the administration of chloral hydrate in a dose of 200 mg/kg causes excretion of free chloral in urine, although in normal dogs there is either no excretion or only a trace of free chloral with such a dose. It is suggested that the difference in levels of elimination of free chloral in urine with such doses of chloral hydrate may be used as a measure of liver damage in humans The excretion of conjugated glucuronic acid cannot be used for this purpose after liver damage by CCl_4 for its excretion is not regular.

After chloral hydrate in doses of 70-200 mg/kg was administered to dogs, free chloral was detected in blood. After hepatotoxic doses of CCl_4, there is increase in concentration of chloral in blood. (Ghosh and Mukherjee, 1941).

8. Lead content is maximum in black hairs, less in brown hairs and least in grey hairs. The hair of married women contains a larger amount of lead, as they use vermilion which is adultered with red lead. Pb is probably present in hairs and other tissues in combination with P. (Bagchi, Ganguli and Sirdar, 1939 *a*).

9. The difference in freezing point between egg-yolk and egg-white of fresh eggs is 0 07°C. If NaF solution which inhibits oxidations and would therefore affect the vitality of the vitellin membrane is introduced underneath the shell, the difference in freezing point gradually diminishes. From this it is concluded that this difference is not due to Donnan Membrane equilibrium but is the result of a

dynamic steady state maintained by the vital activities of the vitellin membrane. (Basu and Mitra, 1940).

10 Food may be contaminated with Pb through solder or enamel. Ghee contains a fair amount of Pb while human milk contains only 0.17 mg/kg. (Bagchi, Ganguly and Sirdar, 1940).

11. On estimating serum phosphatase by Bodansky's method, it was found that 10-20 Bodansky units are present in both obstructive and non-obstructive types of biliary obstruction and that 30 Bodansky units or above are a sure indication of biliary obstruction. (Sehra, Chopra and Mukherjee, 1941).

12. In pulmonary tuberculosis serum phosphatase content increases, but this is lowered by large doses of vitamin C administered orally. (Rudra and Roy, 1942).

13. The acidity of common sesame oil is high. The peroxides present therein destroy carotene and vitamin A. (Ramamurthy and Banerjee, 1949).

14. Neo-stibosan is non-toxic to frog's heart at a concentration corresponding to the initial human dose, and also at double or treble that concentration, but urea-stibamine primarily augments and then depresses the frog's heart at the concentrations slightly lower than the corresponding initial human dose and at a slightly higher concentration is highly toxic.

Histamine has no action on frog's heart at 0.05 mg. per cent, but has a markedly depressant action at 0 12 mg. per cent. The corresponding human doses are estimated to be 0 6 mg. per cent and 1 44 mg per cent. (Basu, 1937 *a*).

15 '*Brihat-Kasturi-Bhusan*', an Ayurvedic heart stimulant, is found to have a very good augmenting effect on frog's heart at 9-18 mg per cent. The corresponding dose for injection to human beings is calculated to be 108 mg. (Basu, 1937 *b*).

16 Application has been made of the Helmholtz principle in tracing the genesis of tetanus curves (Basu, 1937 *c*).

17. An emulsion (5 per cent) of Lifebuoy carbolic soap infiltrated in doses of 1 to 2.5 cc at the site of inoculation of 0.4 mg of cobra venom into pigeons is effectual in saving their lives, even when injected 1½ hours after the inoculation of the venom provided anti-venene is subsequently injected in the latter case. $KMnO_4$ is valueless and even harmful, for it causes sloughing and gangrene.

If the finger of a person is bitten by a cobra, a tourniquet should be applied, one at the base of the finger and another above the elbow, and in the case of a toe being bitten, one at the base of the toe and another above the knee, then 5 per cent soap emulsion should be injected with a sterile syringe — 0.5 to 1cc. at points surrounding the bite — 5 cc. in all could then be given —, and then the anti-venene should be collected and injected. The soap solution should never be given intravenously and is to be used as a first aid measure. Bleeding should be encouraged by crucial incisions with a razor blade. (Ahuja and Brooks, 1945).

18. Carbolic soap emulsion can also detoxicate krait venom *in vivo*. (Ahuja and Brooks, 1948).

19. The physiology of the individual in the tropics has been discussed. (Chopra, 1938).

20. Some biochemical characteristics of cobra and viper's venoms have been reported. (Roy and Chopra, 1938).

Russel's viper venom contains more albumin and less pseudoglobulin than cobra venom. It has 3 6 per cent euglobulin which is absent in cobra venom. It does not contain, like cobra venom, any invertase, diastase or fat-splitting enzymes. It can, like cobra venom, digest fibrin and casein, liquify gelatin and clot milk and is more actively proteolytic than cobra venom). It possesses lecithinase like cobra venom and is either non-haemolytic or slightly haemolytic, while cobra venom is definitely haemolytic.

By a comparative statistical study of the various cardio-vascular tests, it is found that Sneider's Test is the best though it is time-consuming (Telang, 1941-45 and 1948).

22. Actions of neurotoxin, haemolysin and choline esterase, isolated from cobra venom have been studied on heart, blood pressure and respiration. (Sarkar, Maitra and Ghosh, 1942).

23. Comparative study of cobra venom and cardiotoxin in relation to Ca, Na, K and acetylcholine on toad's heart and also in relation to digitaline, saponin and strophanthin on toad's heart and voluntary muscle. (Maitra and Sarkar, 1946).

24. The theory that nutritional oedema is caused entirely by a fall in serum albumin level of blood, is not supported by the following observation·

When the urine of nutritional oedema patients, after removal of electrolytes by dialysis, is injected subcutaneously into albino rats given 5 cc water each, per 100 g body-weight, by stomach tube, they excrete, during the next 4 hours only 47.6 per cent of ingested water, as against 72.5 per cent of ingested water excreted by controls injected with the same amount of normal urine after removal of electrolytes. It appears from the above that there are anti-diuretic substances present in the urine of nutritional oedema patients (Conoor Nut. Res. Lab. Rep, 1949-50).

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PHARMACOLOGY (1938-1950)

Edited by Dr. P. De, F.N.I.

INTRODUCTION

It is difficult to define the scope of a progressive subject like Pharmacology but the following extract from a standard text book is fairly representative· 'The subject of Pharmacology is a broad one and embraces the knowledge of the source, physical and chemical properties, compounding and physiological actions, absorption, fate and excretion, and the therapeutic uses of drugs' Toxicology constitutes a necessary corollary to the effective therapeutic application of drugs.

There are some problems of vital importance to society which a pharmacologist is better able to deal with than workers in other medical sciences These problems include, among others, the influence on health of impurities, preservatives in food, industrial poisoning, drug addiction, and accessory foodstuffs Naturally, therefore, it is apprehended that there will be some encroachment on Physiology and Biochemistry on the one side and on Therapeutics on the other

Dr. Fosdick, President of the Rockefeller Foundation, states "Though no single subject in medicine would seem more important than Pharmacology, which deals with the action of drugs and their uses in disease, this field of medicine is hampered by lack of adequate financial support. This situation is doubtless responsible for the failure of Pharmacology to attract recruits and for shortage of outstanding younger men to fill the professorial chairs which are becoming vacant Larger support is needed not only to promote fruitful research in this important field, but also to improve the teaching on the applied side".

Experimental Pharmacology was introduced towards the end of the nineteenth century. As experimental methods have often led to, and are essential for the introduction of new remedial measures, the importance of a thorough training in experimental work cannot be ignored. Further, special techniques have been developed for standardisation of drugs, chemotherapeutical preparations, etc. *Experimental pharmacology thus entails a training* which is both comprehensive and rigorous.

Research in Pharmacology, really began in India about thirty years back when an independent chair for post-graduate study in the subject was established at the School of Tropical Medicine, Calcutta. Even earlier, sporadic work was done by workers from time to time. Sir Ram Nath Chopra (then Captain Chopra) after joining the Department of Pharmacology at the School of Tropical Medicine trained a band of workers most of whom completed their training by further study under eminent scientists abroad. Almost simultaneously the research workers from other centres in India also received specialized training from abroad and

together with Chopra's associates have subsequently occupied important positions in different Colleges and Universities and made notable contributions in their own lines. Some of these workers have now either retired from the active field or are on the verge of retirement. Due to dearth of qualified men it has been found difficult to fill up the hiatus.

The subject of Pharmacology in India is more recent than other sister sciences, and as such its development does not have a long history. Though the problems facing research workers in this line and the difficulties are immense and multifarious, yet a considerable amount of useful work has been done during the period from 1939 to 1950. These researches have been carried out not only in colleges, universities and special institutions established for the purpose but also by some private laboratories and firms. Attempts have been made to compile this section as thoroughly as practicable within this short space. The collection of materials showing the progress during this long period is also arduous. A task of such magnitude cannot be accomplished with perfection and lapses and omissions are naturally expected to creep in and are inevitable, for which the author expresses his most sincere regret.

The author takes this opportunity of expressing his grateful thanks to all who have helped him in various ways in the compilation of this section.

DRUG LEGISLATION

Central Drugs Laboratory and Its Functions

The establishment of the Central Drugs Laboratory could be traced back directly to the recommendations of the Drugs Enquiry Committee (1930-31) under the Chairmanship of Sir Ram Nath Chopra. It might be recalled that this Committee was appointed by the Government of India to go into the question of adulterated and sub-standard drugs both of foreign and indigenous manufacture, which were being freely offered for sale to the public in India and to recommend ways and means by which this menace to public health could be controlled, and ethical drug trade and scientific medical practice enforced in this country. After a thorough examination of the problem in all its aspects, the Committee recommended that (i) a comprehensive all-India legislation should be passed for the control of the importation, manufacture, distribution and sale of drugs (excluding indigenous drugs) and (ii) a machinery should be set up for the inspection and testing of drugs to ensure conformity to proper standards of purity and strength. With regard to the machinery for drug standardisation, it was further suggested that this should consist of (a) a well-equipped Central Drugs Laboratory with competent staff and experts in various branches of drugs standardisation work and (b) Provincial Control Laboratories working under the guidance of and in close liaison with the Central Drugs Laboratory.

For various reasons, the Government of India could not give effect to the scheme of drug control outlined by the Drugs Enquiry Committee until January, 1937, when the nucleus of the Central Drugs Laboratory (Biochemical Standardisation Laboratory) was established in Calcutta at the All-India Institute of Hygiene and Public Health, as a first step towards a more elaborate organisation to be developed after the passage of the Drugs Act, which was being drafted at that time. In 1940, a Drugs Bill was placed before the Central Legislature and received the assent of the Governor-General in Council in the same year (Drugs Act, 1940). In December, 1945, a set of Regulations known as the Drugs Rule, 1945, was drawn up. In February, 1947, the Government of India, through a Gazette Notification, issued orders converting the Biochemical Standardisation Laboratory into the Central Drugs Laboratory, to undertake all functions envisaged in the Drugs Act, 1940 and the Drugs Rule, 1945 From April 1, 1947, the Government of India decided to enforce the provisions of the Drugs Act, which could not be undertaken earlier because of the war situation and also other difficulties associated with the organisation, equipment and management of the obligations associated with the enforcement of the Drugs Act It will be seen, therefore, that the Central Drugs Laboratory has been functioning only for a few years, though its predecessor, the Biochemical Standardisation Laboratory, had prepared the ground for its activities during the last 13 or 14 years

The Central Drugs Laboratory is now analysing and testing the samples sent to it under provisions of the Act and Rules by Customs Collectors and Courts of Law and also by the Provincial Governments. It also grants certificates of registration for patent and proprietary medicines with undisclosed formulae. Its other activities include standardisation and testing of drugs, pharmaceutical chemicals, insecticides, disinfectants, biological products, chemotherapeutic remedies, etc, according to recognised methods of analysis. Further it has to prepare, maintain and distribute stable standards of strength, purity and quality of drugs, including the distribution of International Biological Standards in India on behalf of the erstwhile League of Nations and the World Health Organisation.

Researches on pharmacological testing of drugs, with a view to elaboration of newer methods of analysis, tests, assay, etc., to suit the climatic and other conditions prevailing in India are being carried on in this Laboratory and a number of papers published embody these results The research projects undertaken by the Central Drugs Laboratory are of the nature of (a) investigations on Indian substitutes for 'Official Drugs' and medicinal foods, (b) studies of the rate of deterioration of potent drugs under Indian conditions, (c) improvements in the existing methods of analysis and standardisation of drugs, and (d) general research on drugs and allied problems.

CHEMOTHERAPEUTIC AGENTS

(a) Studies on Sulphonamide Derivatives

Chemotherapeutic evaluation, toxicological investigation, absorption and excretion studies, bacteriostatic tests, important clinical observations and the study of the mode of action of the drugs have been incorporated in this section

Sulphanilamide, though effective against streptococcal infection, was found to be much less active against pneumococci Efforts to widen its range of activity led to the synthesis of various derivatives. It was observed (Bose, Das Gupta and Basu, 1941) that the compound 2-sulphanilamido-4-methyl-thiazole was just as equally effective in protecting pneumococcus type I-infected animals as sulphapyridine reported by Whitby (1937). While the acute toxicity of this compound in mice was found to be tolerable, chronic toxicity studies carried on rabbits showed the compound to exert a somewhat deleterious effect (Bose, 1941 *a*). The effect noted was a progressive granulocytopaenia, associated with a low grade anaemia. Amir Chand, Taylor and Chitkara (1939) described the effect of M & B 693 in the treatment of pneumonia. The mortality and the days of raised temperature were reduced No serious toxic reactions were noticed with the drug except vomiting More or less similar results were obtained with M & B 693 by Anthony Caplan (1939, 1941), Jekyll (1939) and Taylor and Chitkara (1939).

Further chemotherapeutic study of pneumococcal infection with certain sulphonamide and sulphone derivatives was made (Sen Gupta and Bose, 1946). While bacteriostatic tests showed the compound, p-aminomethylphenyl methylsulphone to be the most active agent against streptococcus, staphylococcus and pneumococcus types I-III, mouse-protection tests did not show such promise. 'Marfanil' (p-aminomethyl benzene sulphonamide) was also found to be inactive *in vivo*.

Among the various sulphonamide drugs that are now being used in bacillary dysentery phthalylsulphathiazole and succinylsulphathiazole are considered to be most potent Both are well tolerated but the latter seems to be less toxic than the former Sulphanilyl benzamide was found (Bose, Sen Gupta and Basu, 1945) to be an equally effective bacteriostatic agent against all the types of dysentery organisms. It has also a low toxicity and further can produce a high effective concentration in the blood of mice in view of its rapid absorption from the gastro-intestinal tract (Bose and Ghosh, 1944 *a*). Its urinary excretion in human volunteers was also rapid (Bose and Ghosh, 1944 *b*). In addition, it was reported to exert a powerful anti-streptococcal action (Bose and Ghosh, 1946 *a*) and was moderately effective against Type I pneumococcal infection (Bose and Ghosh, 1945 *a*).

In a comparative study with sulphathiazole, sulphanilamide and sulphaguanidine, sulphanilylbenzamide was found to be least toxic and to undergo a peculiar selective re-excretion through the coecum and large intestines, not found with the others (Bose, Ghosh and Raksit, 1946). Clinical studies with this compound

demonstrated its therapeutic activity in bacteriologically proved cases of bacillary dysentery (Bose and Ghosh, 1945 *b*). These findings were further corroborated by clinical data from other workers (Swyer and Yang, 1945, Majumdar, Bagchi and Ghosh, 1945; Chakravorti and Misra, 1946).

With a view to have effective chemotherapeutic agents against cholera, typhoid and other conditions not responding to hitherto synthesised drugs, studies on hydroxy-methyl derivatives of different sulphonamides were taken up. The respective formaldehyde condensation products of sulphathiazole (S T F), sulphacetamide (S A F.), sulphanilylbenzamide (S B F), sulphadiazine, sulphamerazine and sulphamethazine were tested *in vitro* againt *vibrio cholerae* (Bose, 1949 *a*) It was found that S.A.F. and S B.F had better bactericidal effect than that exerted by the others Further screening of these two formo-sulphonamides against various pathogens was done in comparison with sulphathiazole (Bose, 1949 *b*). Against *streptococcus viridans* S.A F and S B.F. appeared to be the best bacteriostatic agents; S T.F and sulphathiazole were inactive in this respect S B F. exerted the highest bacteriostatic action against the urinary pathogens — *B pyocyaneus* and *staphylococcus aureus*. Against *E. coli* and *B proteus*, both S A F and S B F. showed similar activity while S T.F and sulphathiazole were found to be inactive even at a concentration of 200 mg per cent All the 'formo' derivatives as well as sulphathiazole exerted powerful bacteriostatic effect against *Sh. dysenteriae (Shiga)* and moderately high effectiveness against *E. typhosa* Against *Sh. sonnei*, S.T F. exhibited lowest activity, while the activity of the rest was of the same order. These findings suggested that extensive clinical trials of the 'formo' derivatives, particularly S.A.F. and S B F might be rewarding

As the basic amino group in the sulpha drugs plays a vital part in producing bacteriostasis attempts are often made to produce their N-substituted derivatives in order to lower their toxicity and/or to enhance their activity In the course of these investigations phthalylsulphacetamide and phthalylsulphanilylbenzamide were prepared (Sikdar and Basu, 1945) and tested by Basu, Mukherjee and Bose (1950) against *E. typhosa* and *S paratyphosa B* They were more active against dysentery organisms and phthalylsulphacetamide showed an increased activity against *B. pyocyaneous*.

Conflicting reports were published by different workers regarding the efficacy of some of the sulpha group of drugs Allen, Sharma, Basu, Jamdar and Panse (1939) reported on the failure of the sulphanilamide therapy in the treatment of coliform and gonococcal infections of the genito-urinary tract. Sanjiva Rao and Ganapathi (1940) appraised the therapeutic value of sulphathiazole in experimental haemolytic streptococcal and pneumococcal (Type I) infections of mice. Experimental evidence showed that this drug was distinctly superior to sulphanilamide and sulphapyridine in streptococcal infection and was as good as sulphapyridine in pneumococcal infection. Sulphathiazole was found by Sanjiva Rao

and Ganapathi (1941) to have appreciable therapeutic effect in experimental *B. typhosus* infection in mice while it was of no therapeutic value in experimental *V. cholerae* and fixed rabies virus infection in mice and vaccinia virus infection in rabbits.

Sulphadiazine, phthalysulphathiazole and sulphasuxidine in the doses used was found by Pasricha, Paul, Das Gupta and Das (1947) to have no beneficial effect in cholera. Chopra, de Monte, Gupta and Chatterjee (1941) observed that sulphaguanidine with 1 g. initial dose followed by 0 5 gm. six hourly for 72 hours effectively reduced the mortality in cholera. In this dose it was not toxic and required less intravenous saline. The mortality rate was 3.84 per cent in culturally positive cases. Gupta, Chatterjee, Paul and Ghosh (1945) reported successful result of treatment of cholera cases with sulphaguanidine. None of the cases treated, developed uraemia The drug also had some vibriocidal and vibriostatic action *in vitro*. Sulphaguanidine-treated cases required less intravenous saline than control ones Sulphaguanidine was also found to be of considerable value in the treatment of cholera by Pasricha, Paul, Das Gupta and Das (1947). The death rate was 3.7 per cent as compared to 7 5 per cent in cases treated exactly on the same lines but without sulphaguanidine (Pasricha, Paul, Das Gupta and Das, 1947). According to Lahiri (1948) both sulphaguanidine and sulphadiazine were beneficial in the treatment of cholera. The mortality rate became lower if the drugs were given before the disease was far advanced. The death rate was higher in children and old persons with both the drugs than with the intermediate age group. On the whole sulphaguanidine gave better results. Seal (1947) found the mortality rate in cholera with sulphaguanidine to be 1.5 per cent against 43 5 per cent in the control The mortality rates in the moderate and severe cases were respectively 1.9 and 2.6 per cent. There were no complications or toxic symptoms.

The anti-haemolytic action of soluseptasine was noted by Roy, Majumdar and Mukherji (1940). It was found to have a retarding action on haemolysis caused by cobra venom, bile salts, saponin and cyclamin. Bacterial haemolysins such as those derived from *Vibrio cholerae* (El Tor) and *Streptococcus haemolyticus* were also neutralized by soluseptasine. Its action on sodium oleate haemolysis was irregular.

In a patient with biliary fistula, De, Datta and Chatterji (1941) observed that the bacteriostatic property of the excreted bile after oral administration of hexamine as well as sulphanilamide was most marked against streptococci. The effect on *B. typhosus* was comparatively less satisfactory and *B. coli* reacted the least.

Braganca and Radhakrishna Rao (1947) produced severe hypoprothrombinaemia in white rat by feeding for a period of three weeks a diet free from vitamin K and containing 1 per cent sulphathiazole. This condition was prevented by the

addition of vitamin K to the diet. Histopathological investigations showed that continued feeding of the drug along with a diet free from vitamin K produced considerable fatty change in the liver and the condition was prevented by the addition of synthetic vitamin K in the diet.

Though sulphanilamide derivatives can exert a sufficient therapeutic action through oral administration, cases where parenteral drug therapy becomes essential and time-saving also occur frequently. Studies on the toxicity and absorption of various soluble sulphanilamide derivatives were therefore undertaken by Bose (1942). From the studies on blood concentration of the drugs, it was concluded that although molar amounts of the drugs were injected under identical conditions, the initial rise, the maintenance and the fall of concentrations in the blood were not the same in each case. This suggested that the toxicity of any soluble sulphanilamide derivative might not depend only on the amount of the sulphonamide theoretically recoverable from it, and that equimolecular doses of different compounds do not produce equivalent concentrations in the blood. The whole problem appeared to be intimately associated with the rate of absorption and excretion of the particular compound

Many theories have been advanced to explain the mechanism of anti-bacterial action of sulphonamide but none has been able to explain all the known facts. The experimental data of Ganapathi and Sanjiva Rao (1940) gave further support to the hypothesis of Trefouel *et al.* that the activity of prontosil was due to the sulphanilamide produced *in vivo* by the splitting up of the azo linkage in the compound Bose (1949 *c*) concluded from his studies that the mode of action of sulphonamides could not be reduced to a unitary concept and that many hurdles might have to be overcome before a correct picture of the same emerged. Studies on the effect of sulphanilamide derivatives on the dehydrogenase activity of resting *E coli* by Bose and Roy (1948) showed that in an anaerobic system, sulphanilamide and its acyl derivatives acted as hydrogen donors, while sulphapyridine was an acceptor of hydrogen This also suggested that the probable mode of action of sulphapyridine might be different from that of sulphanilamide and its acyl derivatives (cf. Dorfman, Rice, Koser and Saunders, 1940)

In explaining the mode of action of anti-dysenteric sulpha remedies Bose, Ghosh and Rakshit (1946) pointed out that a selective re-excretion of the drug through the affected parts of the intestines, after systemic absorption, might play an important role, and that unabsorbability of a drug as advocated by Marshall, Bratton, White and Litchfield (1940) might not always be a sure criterion for effective therapeutic action As a matter of fact, these workers argued that the undisputed efficacy of sulphathiazole, sulphadiazine and sulphanilylbenzamide, could not be explained by Marshall's hypothesis. Unless these drugs were absorbed and re-excreted through the affected tissues, no lasting therapeutic action seemed possible.

Results of three cases of undulent fever treated with sulphonamide were recorded by Bardhan (1943 *a*).

Karamchandani and Sethi (1945) observed that when penicillin was not available, sulphapyridine proved to be the drug of choice in the treatment of gonorrhoea.

Allergy-like toxic effect of sulphathiazole was reported by Gupta (1949). Stematitis, erythema of the skin, extensive desquamation of the epithelium all over the tongue and inner aspects of the cheeks and gums were noticed.

Rao (1949) used sodium sulphacetamide successfully in cases of trachema and warts.

Abdulla and Rohini (1950) reported the efficacy of a patented sulphone compound Formo-cibazol (Ciba) in the treatment of 43 cases of cholera. The results were encouraging, the death rate being only 7 per cent. Bhatnagar and Fernandes (1950) found formo-sulphathiazole and its N'-substituted sodium salt to be powerful anti-bacterial agents. They concluded from their work that an essentially different mechanism of drug action operated in the case of chemotherapeutically active formaldehyde derivatives of sulphathiazole. This was related to their peculiar property of tissue localisation in which their formaldehyde content (11 to 12 per cent) played an important role on account of its interactions with the amino-acids of animal tissues on the one hand and the exo- and endo-toxins of bacterial and virus proteins on the other.

4-hydroxymethylamine-benzene-sulphon-benzamide prepared by substituting N^4-hydrogen atoms of sulphanilyl benzamide was tested against *V. cholerae* by Rao and Basu (1948) and found to exert a bactericidal activity greater than the similarly substituted sulphacetamide derivative and 'formo'-sulphathiazole p-amine-methylbenzene-sulphonamide (Marfanil) has been found by Rao, Chandran and Basu (1949) to be somewhat active against the gram-positive organisms. 'Formo'-derivatives of well-known sulphonamides have been prepared and tested against *V. cholerae* and other pathogens by Bose (1949 *a*, *b*). Formo-sulphacetamide and formo-sulphabenzamide exert a greater bactericidal action than the other compounds.

Khan (1950) assessed the comparative value of sulphadiazine and sulphaguanidine in the treatment of acute bacillary dysentery and reported better results with the former. On an average 7.25 g. of sulphadiazine and 33.33 g. of sulphaguanidine were used for each case.

(b) Studies on Antibiotics

Singh (1949) observed that streptomycin showed no beneficial effect on the occurrence of haemoptysis in tuberculosis. The most marked resolution had been found in soft, nodular and exudative type of infiltration of recent origin. Consoli-

dation and confluent infiltrations did not show the same response. There was no regression of fibre-caseous lesions.

Streptomycin was tried successfully by Narain and Kalra (1950) in 18 cases of tick-borne relapsing fever of Kashmir.

Gokhale and Ranade (1950) suggested that for prophylactic treatment in syphilis streptomycin should replace penicillin as streptomycin acted on all the venereal diseases.

Treu (1950) reported successful treatment of 7 cases of typhoid and para-typhoid fever with chloromycetin. There was no relapse and no unpleasant side-effects were observed in any of these cases, not even when it had been combined with full doses of quinine Its effect on Salmonella appeared to be bacteriostatic rather than bactericidal.

In the treatment of cholera with chloromycetin Chaudhury, Ghoshal and Rai Chaudhury (1950) observed complete disappearance of vibrios within 24 hours of starting the treatment. But clinically there was no improvement in the death rate. *They suggested that it might be useful as a phophylactic agent.*

Sokhey and Habbu (1950) observed that aureomycin and chloromycetin were effective in treatment of experimental plague when given by the mouth.

Patel, Banker and Modi (1949) reported beneficial results with aureomycin in four culture positive typhoid fever cases Similar effect of aureomycin in *typhoid fever had been obtained by Bhattacharjee (1949). Krishnan, Chaudhuri,* Chakravarti and Rai Chaudhury (1950) tried aureomycin in the treatment of typhoid, typhus, cystitis and pertussis. Though its efficacy in typhoid fever appeared to be doubtful it proved to be a very valuable and effective drug for treatment of scrub typhus Aureomycin was also noticed to effect a rapid and complete cure of cystitis due to *B coli* and *S enteritidis*. It was useful in treatment of pertussis Panja, Gupta and Banerjee (1950) observed a prompt and dramatic improvement immediately after the administration of aureomycin in a case of Pemphigus foliaceus.

Wahi (1949) reported penicillin to have beneficial effect in case of tetanus.

Attempts to search for possible antibiotics from higher plants were in progress during the period covered in this review. The anti-bacterial principle present in the roots of *Moringa pterygosperma Gaertn* was isolated by Rao and George (1949). Pterygespermin also inhibited *the growth of many fungi.* Cysteine and thiosulphate, the two sulphydrhl reagents, had no effect on the activity of Pterygespermin. In the presence of small amounts of nucleic acid the activity of Pterygespermin was found to be considerably increased, thereby suggesting its therapeutic importance George, Pandalai and Venkataraman (1947) screened about a hundred of the more important Indian medicinal plants for anti-bacterial substances. They observed that the aqueous or alcoholic extracts of

several of these plants possessed marked anti-bacterial properties against test organisms, such as *S. aureus* and *E. coli*. George and Pandalai (1949) reported the results with 90 more plants. The distribution of the antibiotic principles in the various parts of the plants was found to be uneven and seasonal variations were also marked in some cases. In a further study Mitra, Chandran and Rao (1949) noticed complete inhibition of growth of many organisms with extracts from 11 plant species.

Dhayagude and Banker (1949) reported result of treatment of 24 cases of typhoid fever, all bacteriologically positive, with Bacteriophage. Intravenous 'phage therapy appeared to be sufficiently promising to warrant further trial with type-specific Vi 'phage, either alone or pooled with an 'O' 'phage An unselected stock bacteriophage administered to typhoid patients orally had, however, no therapeutic effect.

(c) ANTIMONIALS AND OTHER ANTI-KALA-AZAR COMPOUNDS

The commercial preparation of all organo-metallic compounds is fraught with difficulties as evidenced by many reports of variation in toxicity between samples of the same product. It is also not exactly known what actually determines the toxicity of organo-pentavalent antimony compounds. In order to determine the factors for such toxicity, a large number of samples of Urea Stibamine prepared under various conditions, as well as the di-ethylamine and tri-iso-propylamine salts of p-aminophenyl stibonic acid were prepared and subjected to both chemical and biological tests in white mice It was found by Bose, Ghosh, Mitra and Dutta (1946) that the toxicity of the Urea Stibamine preparations did not depend solely upon the total antimony content, but was influenced by the presence of antimonious acid. In the case of the secondary and tertiary amine salts, the purity of the amines appeared to be the controlling factor.

Antimony oxides are known to form complex compounds with aliphatic polyhydric alcohols and their aldehydo- and acid derivatives. Such solutions are soluble in water, are of low toxicity and are fairly stable and non-irritant when administered intramuscularly. Since, as an organo-mettalic compound, Antimony(v)-guconate (which is being used in kala-azar) might be liable to variation both in chemical and biological properties, preparations made carefully were assayed, so as to lay down suitable standards for the same. Tests for identification, purity, reaction, estimation and biological assay for undue toxicity and therapeutic potency had been laid down (Bose and Ghosh, 1949). The LD50 of a pure preparation was found to be 2 8 mg. of antimony per g. of body weight of mice (13-15 g.).

Sodium antimony(v)-gluconate was tried in 50 cases of kala-azar, of which 48 showed evidence of immediate clinical cure. Two cases of dermal leishmaniasis showed rapid and well-marked improvement The immediate cure-rate (96 per cent) was closely comparable to that obtained with the best of the pentavalent

antimony compounds (Sen Gupta and Chakravarty, 1945). Napier and Sen (1940) found diamidino-stilbene to be therapeutically effective in the treatment of kala-azar. A cure was effected with a total dose of 1g per 100 pounds of body weight Napier, Sen Gupta and Sen (1942) from the results of their trial of 4:4-Diamidino stilbene (Stilbamidine) in the treatment of ordinary cases of Indian Kala-azar observed that its results compared very favourably with those produced by Neostibosan, the best drug hitherto used. The results obtained in the *treatment of antimony resistant cases were almost as favourable as those in* ordinary cases On the weight basis diamidino stilbene appeared to be at least 4 times as effective as neostibosan. There was however no evidence of its being of any value in the treatment of dermal and cutaneous leishmaniasis

Napier and Sen Gupta (1943) observed no serious or alarming reactions in treating kala-azar by intravenous injections of Pentamidine isothionate (M. & B., 800). It proved fairly satisfactory both in ordinary and 'resistant' cases. Sen Gupta (1944) on further trials with this drug concluded that it possessed some curative action in Indian kala-azar. It showed a higher relapse rate than found with Stilbamidine and was inferior to the best pentavalent antimonials as curative agent in Indian kala-azar.

4,4'-'-diamidino-diphenyl-ether (Phenamidine) was also found to have a fair curative action in Indian kala-azar. The results obtained, however, were inferior *to those observed with the most effective pentavalent antimony compounds or* with diamidino-stilbene (Sen Gupta, 1945).

The effect of urea stibamine on *T evansi* infection in rats was recorded by Dutta and Ghosh (1945). Mukerji and Dutta (1945 *a*) studied the effect of urea stibamine and neostibosan on albino rats infected with *Trypanosoma equiperdum.*

Sodium antimony gluconate (Stibatin) had been reported by Chakravarty (1945) to be a specific drug in the treatment of kala-azar. It had a low toxicity compared to other pentavalent antimony compounds Sen Gupta (1945) also reported that 4:4'-diamidino-diphenyl-ether was relatively non-toxic and it did not produce serious complication during the course of treatment or any sequelae and it could be used in selected cases of kala-azar, such as those exhibiting intolerance to antimony or those complicated with grave pulmonary conditions like pulmonary tuberculosis.

Sodium antimony gluconate (Stibanate) with pedunculine was tried by Sanyal (1947) in the treatment of kala-azar. In all cases the temperature came down to normal after the third injection and Chopra test became negative after the sixth injection. The addition of Pedunculine to the pentavalent antimony gluconate (stibanate) gave a synergistic action to the antimony salt.

Hazarika (1949) tried pentamidine isethionate in 55 cases of Indian kala-azar, all being L.D. bodies positive in the spleen puncture material. 10-15 injections

were given intravenously or intramuscularly in doses varying from 0.75 mg to 2 mg per pound of body weight. The reactions after intravenous injections were very much less than those with stilbamidine and no neurological toxic manifestations could be noticed. The relapse rate was 38.09 per cent.

Methyl glucamine antimoniate was tried in kala-azar by Sen Gupta (1950 *a*) who found the drug to be well tolerated and largely successful. An average effective total dose was also worked out by him.

Urinary excretion of antimony after intramuscular injection of methyl glucamine antimoniate was studied by Chakravarti and Sen Gupta (1950) in 3 cases of Indian kala-azar. It was found that on an average approximately $\frac{1}{4}$th of the antimony dose was excreted in 2 hours and $\frac{3}{4}$th in 12 hours In 48 hours approximately 90 per cent of antimony was excreted in the urine. After 48 hours antimony could not be detected in the urine

Sen Gupta (1950 *b*) tried Hydroxystilbamidine (4 4'-diamidine 2-hydroxystilbene) diisethionate in 42 cases of Indian kala-azar It compares very favourably with older drugs like urea stibamine, neostibosan, stilbamidine, etc. It can be given intramuscularly in 10 per cent solution in 2 per cent procaine. No effect on post-kala-azar dermal leishmaniasis had been noticed.

(d) ARSENICALS, MERCURIALS, ETC.

Carbarsone, p-ureido-phenyl arsenic acid had been found to be useful drug in the treatment of chronic amoebiasis, though it is not accepted as effecting a complete cure. In view of the reports that the corresponding trivalent arsenic compound, p-ureidophenylarsenoxide, exhibited greater amoebicidal activity even *in vitro* it was prepared by the sodium bisulphate reduction of carbarsone and subjected to thorough acute and chronic toxicological tests by Bose, Bose and Ghosh (1950). The reduction product, however, proved to be more toxic to mice and hence appeared unsuitable for chemotherapeutic use in human beings.

Chopra, Basu and Sen (1939) reported on the incidence of rat-bite fever in Calcutta and appraised the result of its treatment with arsenical compounds.

The bactericidal and fungicidal action of organic mercurials with special reference to the dermatomycoses was examined by Byrne and Croxon (1944). A high bactericidal potency was observed by them with phenylmercuricchloride and phenyl mercuricacetate. These have rapid fungicidal effects in high dilutions.

Sulphanilamide derivatives were used by Joseph (1946) in cases of Tropical Eosinophilia with no apparent benefit. Satisfactory results were obtained with aromatic organic arsenicals The association of cutaneous manifestations, gastrointestinal symptoms with the syndrome of Tropical eosinophilia was recorded Splenitic enlargement, lymphadenopathy, cutaneous manifestations and gastrointestinal symptoms were commonly observed in children. Sensitivity to histamine was noted in 12.5 per cent of cases.

Chopra and Sundar Rao (1939) observed that Soamin (atoxyl) and Fouadin (stibophene) gave somewhat satisfactory results against filariasis amongst a large number of drugs tried by them.

(e) Studies on Antimalarials

The acidity of a solution of quinine hydrochloride is often responsible for the necrotic action of the drug. Further, sterilisation of this acid salt solution partly converts it to quinotoxin which lowers the therapeutic efficacy of the alkaloid A systematic study was made from these aspects by Basu, Majumdar and Bose (1940). It was found that no appreciable change in physical or chemical properties of the quinine salt in solution could be detected when it was exposed to either diffuse sunlight or to various bands of the spectrum. The colour of the solution however changed from light yellow to light brown or reddish brown. The maximum photo-chemical effect was found near the blue range of the spectrum The exposure to light did not promote formation of quinotoxin After shortage for one year the quinotoxin content of a solution of quinine dihydrochloride sterilised by heating at 100°C for ½ hour increased only to 3 per cent over the original content of 2 8 per cent. Such an exposure, however, lowered the toxicity of the alkaloid to *Paramoecium caudatum* Further paramoecium studies showed that when a quinine solution was adjusted to a pH of 6 6, it exerted an enhanced lethal action on the paramecia.

In the course of investigations on acridine derivatives as antimalarials Basu and Das Gupta (1937, 1939) prepared the dihydrochloride of 2-chloro-5 (4-diethylamino-1-methylbutyl-amino-1-methoxy-acridine (Mepacrine hydrochloride) and 2-chloro-5(4-diethylaminobutyl-amino)-7-methoxy-acridine and their toxicity and lethal action on paramoecium caudatum were studied (Basu and Bose, 1941). The paramoecium studies showed the lethal action of the latter to be greater than that of the former, thus suggesting a high chemotherapeutic activity Studies undertaken by Siddons and Bose (1944) on monkeys, infected with the highly virulent strain of *Plasmodium knowlesi*, showed that the butyl acridine compound was highly effective as an antimalarial agent However, there were some relapses. Clinical studies on human malaria and intestinal giardiasis also demonstrated its efficacy in doses lower than that of Mepacrine hydrochloride (Bose, Ghosh and Rakshit 1944 *a*)

Atebrin was found invaluable in the treatment of Giardia infection with doses of maximum 9 tablets by Chopra and Das Gupta (1939 *b*), but it had no action on the intestinal flagellates, e.g. *Chilomastix*, *Trichomonas* and *Enteromonas*.

The gametocidal assays of certain substituted quinoline and acridine derivatives were made by Bose and Rakshit (1944) on pigeons infected naturally with Haemoproteus columbae. The compound 6-methoxy-8-(3-diethylamino-propyl-amino)-quinoline, called 'propyl quinoline', was found to be an effective gameto-

cide; while the compound 2-chloro-7-5-(4-diethylamino-butyl-amino)-7-methoxy acridine also possessed some specific action in freeing the blood from parasites. The sulphonamide derivatives of both acridine and quinoline were ineffective. The findings suggested that the replacement of a chlorine atom by a sulphonamide group in both the rings is associated with a dystherapeutic effect so far as the gametocidal activity is concerned In a further study with 8-amino-quinoline-bi-guanide derivatives for gametocidal activity against *Haemoproteus columbae*, the compound N[1] (6 methoxy 8-amino quinolyl) N[5]-p-methoxy phenylbiguanide was found to exert a definite gametocidal activity though lower than that of the 'propyl quinoline' (Basu, Sen Gupta and Bose, 1950).

In studying the antiseptics of acridine series, Singh and Chowdhuri (1948) and Singh, Chaudhuri and Singh (1948) recorded the effect of changing chlorine atom in N-substituted 3-methoxy-9-amino-acridine from position 5 to 7 and 8 and also noted the effect of various substituents and loading of the terminal N-in the dialkylamino Alkyl side chain of type -$NH(CH_2)_4$ at position 9 in 3-methoxy-5-chloro-9-amino-acridine (Singh and Chaudhuri, 1947).

Treatment of relapsing and chronic cases of malaria is still a baffling problem (Bose, 1947). During the study of the antimalarial activity of 2-chloro-5-(4-diethyl amino-butyl amino)-7-methoxy-acridine on simian malaria by Siddons and Bose (1944) it was observed that relapses of malarial fever, which usually occurred after treatment were prevented in those monkeys which had any subsequent injection of Mapharside. Based on these findings a number of relapsing cases of human malaria were studied according to a mode of treatment which consisted of the use of schizonticides during the fever period, and an early administration of a course of sulpharsphenamine (B P.) thereafter. A total of 20 cases were so treated by Bose and Ghosh (1946 *b*) and no relapse had been observed in any one of these cases, which underwent a course of six injections or arsenicals.

Lowe (1944) reported quick control of fever in *P. vivax* infection when quinine was administered after one injection of neoarsphenamine. Das Gupta and Siddons (1944) tested organic arsenicals in the treatment of simian malaria but these did not give satisfactory results. Except with very large doses of 'N.A B.' parasiticidal action of the drug was not much in evidence.

Observations on the use of plasmoquine in pregnancy with reference to foetus were made by Dikshit (1939). Plasmoquine given to animals in doses equivalent to therapeutic dose in man over a period of one week did not influence the activity of non-pregnant uterine muscle. When given in toxic doses to the mother it did not seem to filter through the placenta in sufficient concentrations to influence the heart action of the foetus. The quantity of drug passing through the placental barrier was not very large Dikshit (1941) studied the question of malarial immunity in the rhesus monkey and discussed the role played by 'cellular' and 'humoral' agencies in immunity in monkey malaria. Immunity was retained

when 80 per cent of blood of immune monkey was withdrawn and replaced with normal non-immune blood and also immunity was acquired when more than 70 per cent of the blood of normal monkey was withdrawn and replaced with 'immune blood'. When blood of an infected monkey was replaced with 'immune blood' the infected monkey got rid of the infection and passed into a stage of chronic infection. It was suggested that the cellular agencies play an important and direct part in malarial immunity, whilst the humoral agencies probably play an indirect role by stimulating the cellular mechanism.

Chopra and Das Gupta (1939 *a*) used M. & B. 693 (2-sulphanilylamino pyridine) in ape malaria and observed that it was capable of destroying the monkey plasmodium (*Plasmodium knowlesi*) in dosage which was even less than what was regarded as proportionate dose for a monkey as compared with that of a man Sulphathiazole (M & B. 760) was tried in monkey malaria by Dikshit and Ganapathi (1940). Sulphathiazole administered to monkeys infected with *Plasmodium knowlesi* caused disappearance of the parasite from the peripheral blood in moderate infection with doses as small as 0.5 g once orally Radical cure was obtained with a dosage not larger than a total amount of g given over a period of 3 days. No toxic symptoms were observed. The therapeutic value of three thiazole derivatives of sulphanilamide was appraised by Patel (1944) in experimental *P knowlesi* infection in monkey. (i) N^1-methyl-sulphathiazole with dose of 1 g caused disappearance of parasites from the peripheral blood, but relapses occurred, (ii) 2-N^1-sulphanilamido-5-ethyl thiazole and (iii) 2-N^1-sulphanilamide-5-isopropyl-thiazole with doses as 0 5 g. caused disappearance of parasites from the peripheral blood. Radical cure was produced by drugs (ii) and (iii) with doses 1 and 0 5 g respectively No toxic symptom was produced by their oral administration Das Gupta and Siddons (1934 *a*) obtained encouraging results with mepacrine hydrochloride (B P) against *Plasmodium relictum*

Chopra, Hayter and Sen (1939) tried M. & B. 693 (sulphapyridine) in Indian strains of malaria and found that in 4 g. doses daily for 5 days the malarial parasites disappeared from peripheral blood and controlled symptoms of disease. Both asexual and sexual forms of *P vivax* and asexual form of *P falciparum* were destroyed. In smaller doses recrudescence occurred in a fortnight. In their opinion it possessed anti-malarial properties in Indian strains of malaria Prontosil was tested in Indian strains of malaria by Chopra, Das Gupta, Sen and Hayter (1939) It had in ordinary therapeutic doses (3 to 4 g daily for 5 days) an undoubted action in causing disappearance of malarial parasites from the peripheral blood and in controlling symptoms of the disease. It destroyed both the asexual and sexual forms of *P vivax* and *P. malariae* and only asexual form of *P. falciparum*. It had no action on the crescents. Its action on *P. malariae* appeared to be comparatively slower and less potent. In smaller doses (1.5 to 2 g. daily for 5 days) the symptoms of the disease abated and the parasites disappeared from

the peripheral blood but recrudescence of the disease occurred within a fortnight. It possessed mild antimalarial properties and the authors considered it to be worthy of trial when other antimalarial drugs were not available or were contra-indicated. Chopra and Basu (1939) observed that prontosil in heavy doses failed to prevent the development of crescents in *A. stephensi*. A high percentage of gut and gland infections was observed even after the administration of 40 tablets of this drug

M.3 was tried by Chopra, Roy, Hayter and Sen (1940) in the treatment of malaria. The drug M.3 introduced by the Italian Biochemical Institute of Milan, was said to consist of iron, manganese and extract of spleen It proved ineffective both in the treatment of malarial fever and in preventing relapses even after the patient was treated with quinine, atebrin or plasmochin Neither did it improve the general condition nor reduce the size of the spleen.

Haffkinine or acriquine (atebrin-like compound), an acridine derivative prepared in India was found by Chopra, Hayter and Sen (1940) to be an effective drug in the treatment of Indian strains of malaria. It was effective in doses of 0.1 g. three times a day, the course lasting for 5 days. It appeared to behave in the same way as atebrin Chopra, Hayter, Sen and Talukdar (1940) also tried crinodora (Palusan) in Indian strains of malaria with success It was effective in doses of 0.1 g. three times a day, the course lasting for 5 days making a total of 1.5 g. of the drug for the cure. It was found to have no action on crescents. It behaved exactly in the same way as atebrin.

Heilig and Visweswar (1942) reported that quinine had diuretic effect in massive doses in malarial sub-acute glomerulo-nephritis, nephrotic type and in general anasarca in an old afebrile malaria.

Gupta and Ganguli (1944) investigated the effect of quinine and stilbamidine (M. & B. 744) on the reticulo-endothelial system as measured by the congo-red index They observed that quinine, in therapeutic doses, increased the efficiency of the reticulo-endothelial system, while unchecked malarial infection depressed the reticulo-endothelial system, the degree of infection varied directly with the intensity of infection When malarial infection was controlled with quinine was found to be more than double the rise of efficiency with quinine alone. Stilbamidine (M. & B. 744) did not increase the reticulo-endothelial efficiency.

Quinacrine was also given parenterally in malarial fevers by Basu Mullick and Gupta (1947 *a*). The relapse rate was 11.6 per cent out of a total of 6497 cases and only two cases showed major mental symptoms. Paludrine was given intravenously by Basu Mullick and Gupta (1947 *b*) in malaria with good results. There was complete absence of any toxic symptoms.

Bose, Mukherjee and Chopra (1939) in their survey of the quality of quinine preparations in Indian Hospitals and Dispensaries revealed the unsatisfactory

state of affairs that existed in the country regarding the importation, sale and distribution of an essential remedy for malaria.

Mukerji, Ghosh and Siddons (1942) from their investigations in the laboratory and in the clinic of the total alkaloids isolated from *Alstonia scolaris* (N. O. Apocynaceae) and also of a tincture made from the powdered bark observed that, contrary to the earlier reports, *Alstonia scolaris* had little or no demonstrable action in malaria induced in monkeys or naturally occurring in human patients. It could not, therefore, be recommended as a substitute for quinine and other cinchona alkaloids. The alcoholic extract of the defatted nuts of *Caesalpinia bonducella* when fed in a dose up to 400 mg per g body weight failed to arrest the normal multiplication of *P gallinaceum* in domestic fowls. Moreover the parasite showed no change in morphology (Mukerji, Ghosh and Siddons, 1943).

Ray (1948) after careful trials with paludrine in a tea estate concluded that it was an effective prophylactic and suppressive in both B T. and M.T. malaria in a dose of 0 3 g weekly or 0.1 g. twice weekly at spaced interval Chloroquine is equally effective in doses of 0.25 g. of base once weekly. No toxic reaction with either drug was noticed

Satisfactory results in the treatment of 50 cases of malaria with chloroquine (SN 7618) were obtained by Chaudhury, Rai Chaudhuri and Chakravorty (1948). The drug is effective in terminating an attack in a shorter time compared with quinine, mepacrine or paludrine. 20 per cent of cases, however, relapsed.

Gross (1949) observed the utility of progesterone in the treatment of malaria. He tried in 34 cases and there was one relapse. Clinical facts indicated the possibility of an excess of oestrogens being concerned in the pathogenesis of malaria This excess could be counteracted by progesterone. Progesterone resembles the male hormone and influences the adrenal gland which is involved in serious cases of malaria Therefore, corpus luteum extract with quinine or mepacrine in the treatment of acute malarial attack is suggestive.

55 cases of malaria had been treated with cam-aqi by Chaudhury and Chakravarty (1950) with satisfactory results. Out of 26 cases kept under observation from 1 to 8 months there had been 8 cases of parasitic relapses and one of clinical relapse.

Das Gupta and Siddons (1943 *c*) studied the action of different brands of atebrin in human and simian malaria Of the six monkeys (S. rhesus) infected with a strain of *P. knowlesi*, which is so virulent that infection is invariably fatal if untreated, 4 treated with atabrine (Winthrop) and 2 treated with mepacrine hydrochloride (Bengal Chemical & Pharmaceutical Works Ltd.) survived the primary infection with this parasite. Both drugs had a destructive action on the forms of the parasite occurring in the peripheral blood Relapses invariably occurred after three daily doses of 0.025 g. or six daily doses of

0.0125 g In four out of five animals, such a relapse showed a secondary peak, followed by a natural decrease in the number of parasites leading to a chronic or latent phase. They also recorded the results of these in human malaria.

Paludrine was tried in the treatment of human malaria by Das Gupta, Lowe and Chakravarty (1945). It had an action in malaria comparable to that of quinine and atebrin, the fever due to *P falciparum* and *P. vivax* being promptly controlled and the blood becoming negative In quartan infection the effect was relatively poor.

Basu Mallick (1948) found Paludrine to be a causal prophylactic in preventing malaria.

Basu Mallick and Gupta (1947 *b*) suggested the use of Paludrine by the intravenous route in malaria when oral administration is not possible, and reported good results by this method. The same workers (1948) also used Paludrine in 2% solution in normal saline intravenously in 191 cases of malaria in doses varying from 4 to 7 tablets (0.1 g per tablet), with a mean suppression rate of fever of 74.5 per cent in 12 hours after the injection No toxic symptom was observed The mean relapse rate was 25 32 per cent.

Deshmukh (1947) suggested combination treatment of malaria with quinine, mepacrine and penicillin in acute and relapsing cases.

Quinacrine was given parenterally in malarial fever by Basu Mullick and Gupta (1947 *a*) with successful results, only two cases of major mental symptoms were noticed In the opinion of the workers, this method is a very effective weapon in our hands to fight malaria.

Brahmachari (1944) stated that Berberine sulphate liberated malarial parasites into peripheral circulation from the internal organs and thereby brought the malarial parasites in close contact with quinine in the peripheral circulation.

Alstonia acbolaris was found by Das Gupta, Siddons and Chakravarti (1944) to exercise no synergistic action on quinine in treating malaria. Rai (1946) working on *Enicostema littorle* Blume (Chhota chiraits) observed that it cannot compete with cinchona alkaloids in its effectiveness.

(f) Drugs Used Against Plague, Filariasis, Etc.

All our efforts to find a successful remedy for the treatment of Plague have been practically baffled. The chemotherapeutic agents and antibiotics also have failed to produce the desired result, though only in cases of bubonic plague some of these have proved to be effective to a limited extent.

Sokhey and Dikshit (1940) reported good results with sulphathiazole in Bubonic Plague. The comparative effect of treatment of septicaemic and non-septicaemic cases of plague with serum, sulphapyridine and sulphathiazole was described by Wagle, Sokhey, Dikshit and Ganapathy (1941). The mortality rate in treated

cases, as reported by them, was lower than that of controls. Successful treatment of a case of plague with sulphapyridine had been recorded by Chopra, de Monte and Chatterjee (1941). 2 N[1]-sulphanil-amidothiazole had been found to be effective in experimental plague infection in mice by Sokhey and Dikshit (1940). Wagle (1944) in a later communication noted that in bubonic plague the results of sulphadiazine treatment were slightly better than those of sulphathiazole Sulphadiazine also maintained a higher blood concentration than sulphathiazole with the same dosage No serious toxic reactions were encountered with any of these drugs, even the incidence of mild reactions was low. Sulphadiazine on the whole produced fewer toxic reactions.

The effect of sulphadiazine and penicillin on experimental animal plague was investigated by Gupta, Panja and Chatterji (1946) Wagle (1948) discussed the remedies used in the treatment of bubonic plague and considers streptomycin to be the most effective drug.

Ghosh (1950) discussed the usefulness of streptomycin and sulphadiazine in the treatment of plague on the basis of treating 155 cases.

Lithium Antimony Thiomalate has been found to produce some encouraging results in the treatment of early cases of filariasis, Raju Ayyar (1949).

Chopra and Sundar Rao (1939) tried a large number of drugs in the treatment of filarial infection The effects of organo-metallic compounds, prontosil and its derivatives, oil of chenopodium, cobra venom, Russell's viper venom, etc. were compared. No drug was found to have satisfactory anti-filarial properties Soamin and Fouadin gave somewhat satisfactory result amongst all these drugs

Successful results had been obtained in the treatment of gonococcal infection with some of the chemotherapeutic and antibiotic drugs Rajam Rangiah and Masilamani (1939) reported that in the treatment of gonorrhoea and other venereal diseases with sulphonamide compounds the best results were obtained with massive potentially toxic doses of the drug for a minimum period of three weeks. Uleron was the least toxic and sulphanilamide and prontosil album were the most therapeutically effective drugs of the series. Continuation or otherwise of the treatment on appearance of the toxic symptoms had been discussed.

Chopra, de Monte, Chatterjee and Gupta (1940) in their studies on the treatment of meningococcal and pneumococcal meningitis with diaminodiphenylsulphone glucoside gave a comparative result of treatment with the drug either alone or with anti-meningococcal serum in these conditions and concluded that diamino-diphenyl-sulphone glucoside under the condition of their experiment appeared to be of little value in the treatment of meningococcal meningitis.

In spectroscopic examination of blood in the treatment of meningitis with diamino-diphenyl-sulphone glucoside the appearance of the absorption band at

630 mu and its behaviour on addition of ammonium sulphide were used as a very sensitive method for detecting the presence of methaemoglobin and sulphaemoglobin in blood. It was noted that on administration of diamino-diphenyl-sulphone glucoside for meningococcal infection no sulphaemoglobin was produced. Also it was observed that methaemoglobin began to appear on the administration of the drug only after the cerebrospinal fluid became clear. When the administration of the drug was stopped after the appearance of methaemoglobin in the blood, no toxic effects were produced (Chopra, Seshan and de Monte, 1940).

Sulphonamide was tried by Patel and Naidu (1940) in the treatment of small pox. It reduced mortality in confluent cases by about 30 per cent. It had no influence on the course of small pox except in cases of septic type. The drug had no effect on haemorrhagic cases

Cadmium sulphide was found by Ray, Sen and Das Gupta (1941) to be as useful in the treatment of pulmonary tuberculosis as solganol and sanocrysin. It was considered to be a valuable adjunct of collapse therapy.

A number of Indian brands of iodochlorhydroxy-quinone (Vioform) were tested according to methods laid down in N.N R. 1941 by Pal, Mukerjee, Gupta and Chatterjee (1944) and compared with the standard product of Ciba Company. They differed slightly in chlorine content from the standard product, but agreed closely in melting point, physical characteristics, solubility, iodine and chlorine percentages with the standard. Their toxicities were within safe limits in therapeutic dosages In clinical trials in controlled cases of amoebiasis in hospitalised patients, it gave comparably satisfactory results. Maz Mayer (1940) also recorded satisfactory results of treatment of acute, subacute and chronic cases of amoebiasis with enterovioform (Ciba).

McDonald (1943) reported a case of benign neutropenia treated by sodium pentnucleotide The neutrophil count was significantly higher during and after pentnucleotide therapy.

The use of carbaminoylcholine chloride (Doryl) in angiospasm and obstructive jaundice suspected to be due to impaction of the biliary passage with stone, was suggested by Prasad (1939).

Hardikar, Mohiuddin and Siddiqi (1942) worked on the urinary antiseptic action of hexamine. The concentration of formaldehyde obtainable in urine after oral administration of hexamine was not sufficient by itself to kill or inhibit the growth of organisms such as *B. typhosus* in the urinary tract. Any beneficial effect of hexamine therapy must, therefore, be attributed to the liberated formaldehyde action as a reinforcement to the protective mechanisms of the body already in operation. The idea that the benefit may be due to hexamine itself is, in the opinion of the workers, still less tenable.

IV. HORMONES

(a) ADRENALINE

Bose, Dutta and Mukerji (1942) found from the assay of various samples of Indian and foreign made adrenaline, that adrenaline solution marketted for use deteriorate on storage. Mukerji and Dutta (1945 *b*) later confirmed this. Detailed investigations on the rate of deterioration of solutions of adrenaline hydrochloride and adrenaline tartrate stored under ordinary storage conditions in India were carried out by Chowdhuri and Mukerji (1946) after storage periods varying from four months to approximately four years It was observed that both the liquid preparations deteriorated rather rapidly after the first 10 months from the date of their manufacture Chowdhuri, Roy and Ghosh (1946) in their studies on the stability of adrenaline-procaine solutions and tablets, analysed forty preparations of combined 'Procaine-Adrenaline' of foreign make for their respective procaine and adrenaline contents, using 'standard' and well accepted methods in both cases In all samples, the procaine content was found to be up to the claim whereas the adrenaline content was found to have invariably fallen off This deterioration appeared to be more marked when adrenaline was present in combination with procaine than when present alone. The presence of a stabiliser reduced the chances of deterioration only to a certain extent, but could not do so completely. There was no significant difference in the rate of deterioration between solutions and tablets

Studies made by Bose and Ganguly (1942) with a number of synthetic and natural adrenaline powders indicated no correlation between optical rotary power and biological potency.

Stabilization of adrenaline solution with Sodium bisulphite as described in British Pharmacopoea (1932) was found by Ganguly and Ghosh (1945) to lower its physiological activity It was noticed by Basu, Ganguly and Bose (1945) that solutions of adrenaline exhibited definite changes in optical rotation at room temperature (25° — 30°C) under conditions when no change in pH, or formation of colour or precipitate took place Such solutions showed remarkable losses of biological activity though chemical assay revealed no significant differences Attempts were therefore made to stabilise adrenaline solutions by increasing the optical activity of the solution with the formation of laevo-organic salts of adrenaline It has been found from such studies that adrenaline-1-malate solution keeps its potency for a far longer period than has hitherto been observed with any other salt of adrenaline (Basu, Ganguly and Bose, 1945).

(b) GONADOTROPIC HORMONE AND SUPRARENAL CORTEX

The efficacy of the gonadotropic hormone in the treatment of whooping cough was reported by Venkatachalam and Ratnagiriswaran (1939). After 6-12 injections

of 0.25 cc. of antuitrin 'S' vomiting and whooping stopped completely. Varma and Nagar (1948) observed that the gonadotropic hormone in pregnancy urine affected the gonads in *Barbus stigma*, in the same way as in mammals.

Joseph (1945) obtained satisfactory results in the treatment of hypertrophy of prostate with the oestrogenic hormone, stilboestrol. Oral administration of stilboestrol in doses of 10 mg per day for a period of 10-15 days was found by Ojha and Venkatachalam (1949) to apparently effect clinical and radiological cure of peptic ulcer

De and Montgomery (1947) investigated on a case of feminism and from the biochemical reports of the patient came to the conclusion that the condition was the result of hyperactivity of desoxycorticosterone and reduced activity of corticosterone and dehydro-corticosterone due respectively to hyperplasia of the zona glomerulosa of the supra-renal cortex and atrophy or degeneration of zona fasciculata and zona reticularis The degeneration of the inner zones of the cortex was due to reduced secretion of adrenotropic hormone of the anterior pituitary.

Montgomery and De (1947) described the isolation of oestrone from the urine of a case of feminism by a process of selective adsorption. The authors suggested from the results of their enquiry that pituitary adreno-corticotrophin consisted of two factors, and that each exerts its effect on different zones of the adrenal cortex. Androgen and oestrogen are elaborated in different zones and excessive production of either results from the particular zone which is overactive, or is the main seat of tumour formation.

Vakil (1949) obtained favourable results in the treatment of 125 cases of angina pectoris with testosterone propionate In the treatment of 700 cases of female genital disorders Kirloskar (1949) reported beneficial effect of steroid hormones.

Mahanty, Pabrai and Paramjit (1950) carried on Sperm Test of pregnancy, based on the principle of Galli Mainini, by utilising male toad, *Bufo melanosticus* Schneid, as test animal In a series of 308 samples from 143 cases of pregnancy, no 'false negative' result was obtained. With 92 samples of non-pregnant and male urine and frog saline, no 'false positive' result was recorded. Within a certain limit, a positive correlation exists between the concentration of the hormone in urine and intensity of reaction In the first week of pregnancy and towards the end of the term, in some cases, the reaction was found to be negative. It is a simple, inexpensive and rapid test with a high degree of accuracy.

(c) Insulin, Hypeoglycaemia, etc.

Allinson and Paul (1939) noted that the absorption of insulin was greater from the nasal mucous membrane than from other mucous surfaces.

Bose (1939) studied the effects of protamin zinc insulin and discussed its advantages over ordinary insulin.

Insulin sensitivity among the Indian diabetics was worked out by Gupta and Hameed (1947). They found the relative proportion of insulin sensitive and insulin insensitive types of Indian diabetics is 67 per cent and 14 per cent respectively An intermediate type between these two had been described. In the opinion of the authors the classical description of two types of diabetes (sensitive and insensitive) did not hold good for Indian diabetes.

Bose (1948) investigated the role of vitamin B_1 in the treatment of diabetes. Marked improvement in carbohydrate tolerance of the patient was observed in most cases of diabetes showing vague and indefinite symptoms of vitamin B_1 deficiency. In some cases complicated with neuritis there was a definite relief from pain and the amelioration of the associated symptoms such as weakness, paraesthesia was also marked. No claim was, however, made on the cure of these cases. It was *found that in some cases considerable reduction in dose of insulin could be made* if vitamin B_1 was simultaneously administered Prasad and Prasad (1940) working on the same line observed that the requirement of insulin could be reduced by more than 50 per cent when insulin treatment was combined with vitamin B_1 and C.

Ojha, Purbai and Venkatachalam (1949) observed that aqueous extract of *Ptorocapus marsupium* Roxb on oral administration lowered the blood sugar in rabbits and diabetic patients.

Iyenger (1941) in his work on competition of protein substrates towards proteolytic enzymes noted from the experiments conducted that among the proteins tested, insulin was the most resistant to tryptic attack when other proteins were present in reaction mixture. Proteins which are easily digested by trypsin, appear to offer protection to insulin from enzymic proteolysis

The comparative action of trypsin on amorphous insulin, crystalline-zinc-insulin, globin-insulin and protamine-zinc-insulin was studied by Iyenger (1946 *a*) by following both the inactivation of insulin as well as the increase in non-protein nitrogen. The rates of inactivation of insulin as well as the digestion of insulin protein were found to be in the decreasing order for the above forms of the hormone.

The 'in vitro' action of alloxan on insulin partially inactivated by the action of thioglycollic acid under controlled conditions without appreciably denaturing the protein, was investigated by Iyenger (1946 *b*)

Attempts were made by Iyenger, Biswas and Mukherji (1947) to mix insulin with a casein-hydrolysate (Pronutrin) containing a mixture of simple peptides. [illegible]

De (1946 *a*) investigated the blood-sugar level in decerebrated animals and indicated the levels of transection that would produce hyper or hypoglycaemia in acute experiments.

De (1946 *b*) worked on the cause of hyperglycaemia under general anaesthesia and concluded that the hyperglycaemic effect was due to release of the hypothalamic sympathetic centre from the normal cortical control. Also the cause of hyperglycaemia during and immediately after operation were investigated by De and Datta (1946).

(d) Pituitary

Difficulties in isolation of active glandular principles in our country are due to lack of suitable abattoir facilities, proper preservation and transportation of frozen glands as also low veterinary standards of health of slaughtered animals. Pituitary (posterior lobe) extracts and bile salts are two important therapeutic preparations which require suitable glands for isolation and satisfactory yield. Studies made with a large number of bovine gall-bladders showed that the yield of bile was poor but the bile salts and cholates isolated from them exhibited their characteristic chemical and biological properties (Basu, Bose and Das Gupta, 1940). Similarly the yield of active posterior pituitary powder from bovine pituitary glands was also found to be low, though its potency compared favourably with foreign products(Basu, Bose and Gupta, 1944).

(e) Thyroid

Thyroid gland powders of local and foreign manufacture were assayed for the thyroxine-iodine content by Mukerji (1943 *a*) The values with the Indian samples were generally higher.

V. PEPTONES AND PROTEIN HYDROLYSATE

Studies on the fractionation of primary and secondary proteins were made by Sen and Bose (1944) in an attempt to elucidate the mechanism of anaphylactoid reactions Two types of reaction could be detected with the primary proteoses.

Narayanan and Krishnan (1944) reported that protein hydrolysate, specially made by them, had a definite value in the chronic hypoproteinaemic conditions such as starvation, when given intravenously. It is not superior to serum or plasma in the treatment of traumatic shock unless it is further reinforced with suitable colloids to bring the blood pressure raising quality to the level of serum, it can therefore occupy only a second place in the treatment of traumatic shock.

It was observed by Krishnan, Narayanan and Sankaran (1944) that properly prepared enzymic hydrolysate would be the cheapest and best product for parenteral therapy in inanition. The rationale of therapy in inanition was also discussed by the authors.

Viswanathan (1945) reported that protein hydrolysate had definitely a place in the treatment of cases of infective hepatitis with toxic symptoms. Vomiting

disappeared and appetite was restored very quickly. The liver became normal and sedimentation rate also came down to normal within a shorter period. Better results were, however, obtained in those cases treated early.

VI. RESEARCHES ON THE INDIAN INDIGENOUS DRUGS

Systematic researches on scientific lines on the Indian indigenous drugs were started about the year 1924 at the Department of Pharmacology of the Calcutta School of Tropical Medicine This scheme was taken up by Sir Ram Nath Chopra (then Major Chopra) with the idea of exploring the drug resources of our country to evolve cheap and effective remedies for some of the common ailments The impetus given by the Calcutta School of Tropical Medicine stimulated others to work on this line of investigation. A considerable amount of work was done during this period some of which as e.g on Rauwolfia serpentina, proved to be very useful These investigations not only helped to select the useful drugs from the labyrinth of reported cures but also assisted in finding out Indian substitutes for 'official drugs'.

Chopra, Chatterji and Ghosh (1940) worked on *Boerhaavia diffusa* Linn, and white and red flowered varieties of *Trianthema portulacastrum* Linn, which are used indiscriminately in different parts of India as the Ayurvedic drug Punarnava The former belongs to the family Nyctaginaceae and the latter to Ficoidaceae. Although they belong to two different families, the chemical constituents which may account for their medicinal properties are common Both contain comparable quantities of potassium salts, mainly potassium nitrate, *T portulacastrum* (red) showing the highest percentage of nitrates, and *B diffusa* the least. Both contain the same water soluble active alkaloid Punarnavine, *T portulacastrum* (red) and *B. diffusa* showing similar amounts, while *T. portulacastrum* (white) showing less. The total extractives are the highest in the case of *T portulacastrum* (red) and least in the case of *B. diffusa* The other water-soluble bases, which are comparatively inactive, show similar amounts in all the three plants.

Two saponins were isolated from *Entada pursaetha* Dc. (*E Scandens* Benth) Chopra, Gupta, Chopra and Ghosh (1940) found these to have identical actions and to be almost equally toxic. These saponins were much less toxic to paramoecia and non-toxic to mosquito larvae. The main action was on the haemopoietic system where they caused haemolysis of the red blood cells. A sharp fall of blood pressure was observed in experimental animals after doses of saponins, varying from 0.0005 to 0.002 gramme per kilo body weight The fall was associated with an increase in the volume of the intestines, and to a lesser extent those of the kidneys. The fall of blood pressure might partly be the result of the dilatation of the vessels of the splanchnic area and partly due to the depressant effect on the myocardium. The fall in blood pressure was absent in animals given atropine. The saponins had depressant effect upon the respiratory system and death appeared

to result from respiratory failure They had also inhibitory effect on the movements of unstriped muscles of the intestines and the uterus.

Chopra, Kohli and Handa (1941) found that the essential oil of *Curcuma longa* Linn. had feeble antiseptic properties. It acted as antacid by inhibiting the secretion of gastric juice. It produced a carminative effect and in large doses acted as an antispasmodic.

The insecticidal and larvicidal properties of *Blumea densiflora* Dc and *Artemisia vulgaris* Linn were studied by Chopra, Roy and Ghosh (1940). Their experiments showed that *B. densiflora* did not possess insecticidal and larvicidal properties to any great extent. In this respect *A vulgaris* was a little more effective and yielded nearly the same results as were obtained with pure Kerosene, which itself is a feeble insecticide but a good larvicide Nyjkamp and Swellengrebel (1934) recorded a 33 per cent kill by merely spraying finely powered leaf of *Artemisia maritima* Linn on house-flies. The dry powder of A vulgaris on the other hand, did not act as a contact poison to flies.

Roy, Ghosh and Chopra (1941) reported results of treatment of scabies and pediculosis with pyrethrum.

The comparative effects of the alkaloids of *Rauwolfia serpentina* Benth were investigated by Chopra and Chakraburty (1941). Serpentine was found by them to be the most toxic and ajmaline and serpentinine were about equally toxic on intraperitoneal injections in white mice. Crude extracts of *R. serpentina* produced fall in arterial pressure which seemed to be peripheral in origin. Ajmaline raised the blood pressure in decerebrate animals but lowered the pressure in spinal preparations. This fall in pressure was more marked when the pressure was previously raised and maintained at a higher level by slow continuous intravenous injection of adrenaline Serpentine produced fall in arterial pressure, both in the decerebrate and in the spinal preparations. The effects of serpentine closely followed those of ajmaline on the blood pressure of experimental animals. Neither ajmaline nor serpentine had any narcotic effect in mice. Serpentinine possessed such effects Sedative effects were, however, found with the crude extracts.

Chopra, Bose, Gupta and Chopra (1942) further studied the comparative effects of the alkaloids of Rauwolfia serpentina in experimental hypertension and observed that the extract, total alkaloid and serpentine showed marked hypotensive properties; ajmaline and serpentinine, on the contrary, were hypertensors. In experimental hypertension all of them induced hypotension, serpentine producing the maximum amongst them and serpentine could be considered to be the chief pressure-reducing factor amongst Rauwolfia alkaloids. Ajmaline and serpentinine stimulated, whereas serpentine depressed the intestine. They suggested that the Rauwolfia alkaloids probably acted on the vaso-motor system and also directly on the plain muscle of blood vessels and intestines.

A study of the comparative pharmacological action of the total alkaloids of Rauwolfia serpentina Benth obtained from Bengal, Bihar and Dehra Dun was made by Gupta and Kahali (1943). They found that the extent and the duration of the hypotensive effect produced by the total alkaloids obtained from the roots was in the decreasing order of Bihar, Dehra Dun, Bengal for the three varieties. The Dehra Dun alkaloid had depressant action which was purely circulatory (central, cardiac and vascular). Bihar and Bengal root alkaloids had, in addition, slight sedative action on the central nervous system. All the three produced depression of respiration and dilatation of bronchi Uterus was stimulated by all, the Bengal variety being the most active while the Bihar variety was the least potent. All the three had dual action on intestine-(i) a preponderating parasympathomimetic action and (ii) direct slight depressant action The pharmacological action of total alkaloidal extracts from the Bihar and Dehra Dun roots of Rauwolfia serpentina was described by Kapur (1948). Both the extracts produced a fall in carotid blood pressure, the Bihar variety having somewhat more powerful action Heart was also depressed by both the total extracts nearly to the same extent.

Bhatia and Kapur (1944) investigated the pharmacological action of the alkaloids neoajmaline and isoajmaline from Rauwolfia serpentina Benth They found that both the alkaloids had slight stimulant action on the nervous system, which was followed by well-marked depression. These also had a depressant action on plain muscles of the heart, blood vessels and intestine Both the alkaloids also lowered blood pressure in intact, spinal and decerebrate animals in normal condition and also after experimental hypertension Neoajmaline had a powerful stimulant action and isoajmaline a mild depressant effect on the uterus of rabbit and guineapig. The average toxic dose of neoajmaline was 0 065 mg. per gramme body weight of guinea-pig, isoajmaline was slightly more toxic In any case death was due to respiratory failure.

The hypnotic effect of R. serpentina was investigated by Chopra, Gupta, Bose and Chopra (1943). It was found that the alkaloids ajmaline, serpentine and serpentinine were medullary stimulants and provoked convulsions and anoxaemia. The sedative and hypnotic properties were present mainly in the alcoholic extract, and in the total alkaloids free from ajmaline, serpentine and serpentinine. The hypnotic principle antagonised the medullary stimulation of picrotoxin and had depressant action on the medullary centres. The investigation of the hypnotic effect was extended to the resin fraction isolated from the root of *R. serpentina* obtained from Dehra Dun by Gupta, Kahali and Dutt (1944). From the crude resin of the plant a fraction insoluble in petroleum ether but soluble in alcohol was isolated and was found to be pharmacologically active. In experimental animals (cats, rabbits, rats, frogs and guinea-pigs) it produced a sedative and hypnotic effect very similar to that produced by a standard preparation. The seda-

tive action commenced about 3 to 4 hours after administration and persisted for more than 24 hours. The resin or alcohol soluble fraction did not cause a fall of blood pressure but stimulated the simple muscle of the intestine and uterus.

Gupta, Deb and Kahali (1943) used *R. serpentina* in the treatment of mental disorder. Fifteen patients suffering from various types of mental disorder were treated with standardised extract of the plant. All these patients suffered from insomnia and showed considerable motor and mental agitation. The extract was given three times a day in 30 minims doses or even smaller doses for under-nourished cases. It produced depressant action on the circulation and had cumulative effect. The pulse and blood pressure should, therefore, be carefully observed and the dose controlled accordingly. The sleep ensued 2 to 3 hours after the evening dose and lasted for more than 6 hours. The patients became quiet and behaved more normally, and those suffering from affective disorder showed considerable improvement. The alimentary functions of the patients were stimulated and appetite improved.

Gupta, Roy, Ray and Ganguly (1950) studied the excretion of total alkaloids of Rauwolfia in urine A fluorometric method for estimation of the Rauwolfia alkaloids in urine was devised and urinary excretion in 6 cases studied Maximum percentages of the alkaloids excreted in urine in the free state generally occurred in the sample collected between 1 to 2 hours after the intake of the same. No alkaloid was eliminated within half-an-hour and the drug appeared one hour after ingestion. So long as pressure was being reduced, the excretion of alkaloids was low but as soon as pressure became more or less steady, the excretion generally increased to a high level and a total of about 50 per cent of the dose was eliminated within 3 to 4 hours of the intake of test dose.

De (1943) investigated the pharmacological action of a bitter principle isolated from *Securigera securidaca* (Linn.) Dagen et Dorfler (N. O *Leguminosae*) on the circulatory system and observed that it produced pressor effect on circulation by its direct action on the cardiac musculature and plain muscle of the blood vessels and also partly by its action on the sympathetic nerve-endings. A fall of blood pressure was noted with bigger doses of the bitter principle, which was due to the stimulation of the vagal centre in the medulla.

Grewal and Kocchar (1943) isolated from the seeds of *Luffa acutangula* Roxb. a fixed oil and a saponin which were found to cause vomiting and purging in dogs. In small doses nausea and salivation were produced.

The glucoside Cerberin obtained from *Cerbera odollam* Gaertn was shown by Chopra, Bose, Gupta and Chopra (1942 *a*) to act as a cardio-tonic remedy. It also increased the tone and peristaltic movements of the intestine, which was removed by atropine and potentiated by pilocarpine, suggestive of a parasympathomimetic action. Chopra, Gupta, Bose and Chopra (1942) also investigated the pharmacological action of the glucosides of *Paris Polyphylla* Sm. Of the two

glucosides -paristyphnin was pharmacologically more potent than -paridin The different actions of -paristyphnin were probably due to its direct action on non-striated muscle fibres

Gupta and Kahali (1944) investigated the pharmacological action of Umbellantine, a new alkaloid from *Berberis umbellata* Wall. and *Berberis insignia* Hook F. It had a specific inhibitory action on the growth of Leishmania tropica and was used with success in the treatment of oriental sore It depressed the cardiovascular system. The heart was depressed directly. The blood vessels were directly and also perhaps by stimulation of the parasympathetic vaso-dilator nerve endings. The smooth muscles of spleen, intestine, uterus and bladder were stimulated. The respiration was depressed and bronchi constricted Some of these actions were due to stimulation of the parasympathetic nerve endings concerned.

A comparative study of alkali-soluble and alkali-insoluble fractions of the Indian hemp, *Cannabis sativa* Linn. in carbon tetrachloride and petrol-ether solvents was taken up by Bose and Mukerji (1943) and (1945) The total extract and the alkali-insoluble fraction were found to possess the narcotic properties of the drug as evidenced by the production of ataxia in cats Homotetrahydrocannabinol or an allied compound was possibly the active principle for the narcotic action of hemp The activity of the alkali-insoluble portion of hemp resin was greater than that of homotetrahydrocannabinol. The alkali-insoluble portion probably contained some optical isomers of tetrahydrocannabinol which were more active than tetrahydrocannabinol or its optically inactive homologues The alkali-soluble fractions were found to be devoid of all physiological activities.

Cotarnine hydrochloride, prepared from Indian Narcotine, was subjected to toxicity tests and certain pharmacological investigations by Bose and Ghose (1949). No significant difference in toxicity could be found when compared with a fine sample of Merck's cotarnine salt. Isolated virgin guinea-pigs' uteri were stimulated and such contractions were always released by adrenaline In chloralosed cat with intact central nervous system, cotarnine produced a definite fall in blood pressure but in a spinal cat it caused a distinct but small rise of pressure. This depressor effect was so powerful that even in artificial hypertension the effect was marked. On the contrary adrenaline could not produce a pressor effect on cats, having infusion of cotarnine. These phenomena suggested some sort of antagonism between adrenaline and cotarnine

Chopra, Kohli and Henda (1945) working on some common Indigenous remedies isolated a non-poisonous alkaloid, atisine from the rhizomes of *Aconitum Hetrophyllum* Wall. Intravenous injection of 10 to 12 mg of this alkaloid produced a transient fall of blood pressure It had no antipyretic effect in similar doses. A water-soluble glucoside was isolated by these workers from the seed of *Xanthium strumarium* Linn It was found to be inactive. They also isolated very small quantities of a bitter substance from the whole plant of *Lippia nodiflora*

Rich. This also proved to be inactive. The presence of nitrate possibly accounted for alleged diuretic action of the plant.

Chopra, Kohli and Handa (1945) isolated from *Inula royleana* Dc., the alkaloid roylene having the empirical formula $C_{21}H_{33}O_6N$. The alkaloidal content was about 3 per cent. A fall of blood pressure and stimulation of the tone and rhythmic movements of intestines on intravenous administration of this alkaloid to urethanised animals were observed. It produced no action on free living ciliates and had no bactericidal action.

An active constituent of glucosidic nature, which appeared to be of very low toxicity in frogs, rats and mice, was isolated from *Vanda roxburghii* R. Br. by Gupta, Roy and Sen Gupta (1946) It produced a stimulant action on organs innervated by cholinergic autonomic nerves, and to a small extent also acted directly on some involuntary muscles. The carotid blood pressure was appreciably lowered even in low doses. These effects appeared to be due to (i) stimulation of cholinergic nerve endings in the organs concerned, and later (ii) stimulation of the vaso-constrictor nerves through the centre; they also appeared to be due, to small extent to (iii) direct stimulation of involuntary muscles.

An active constituent of glucosidic nature isolated from *Daemia extensa* R. Br. (Syn. *Pergularia extensa*) was investigated by Gupta, Roy and Dutta (1946). It was toxic to white mice, guinea-pigs and cats and had a stimulant action on the involuntary muscles and a pronounced effect on the circulatory system, raising the arterial blood pressure appreciably The uterus was powerfully contracted like pituitrin. These effects appeared to be due to (i) the direct stimulation of involuntary muscles and possibly (ii) the stimulation of post-ganglionic cholinergic nerves in the structures concerned

The pharmacological action of 22 indigenous drugs reported to be useful in uterine disorder was investigated by Kapur (1948). Of these *Gossypium herbaceum, Helleborus niger, Peganum harmala, Plumbago rosea, Rauwolfia serpentina, Rubia cordifolia, Nerium odorum, Nigella sativa, Ruta graveolens* and *Sesamum indicum* produced stimulant action on guinea-pigs' isolated uterus. Out of these *G. herbaceum, H. niger, P. harmala, P. rosea, R. serpentina* and *R. cordifolia* were found to stimulate the uterus of women in puerperium. The oxytocic activity of these six was biologically compared with oxytocin and in descending order of their activity the drugs could be arranged as — *P. harmala, R. serpentina, G. harbaceum, P rosea, H. niger* and *R. cordifolia.*

Gupta, Kahali and Ganguly (1942) worked on the anti-haemolytic property of *Vitex peduncularis*. It increased the osmotic resistance of the red blood cells of animals and inhibited haemolysis by saponin, acid, cobra venom and bile salts. It was absorbed when given intramuscularly, the maximum effect being attained within two hours, the injection was apparently without any toxic effect. Absorption through the alimentary tract might take place but the rate was extremely slow and

depended on the size of the dose. It was assumed that the action was on the red blood cell surface.

Measham (1940) tried *Vitex peduncularis* in the treatment of blackwater fever and found it to be of definite value.

Cassia fistula was used by Venkatachalam and Ratnagiriswaran (1941) in the treatment of Blackwater fever The cure rate worked out to be 96 3 per cent, urine cleared in 3 or 4 days, after giving 12 — 16 fl. dr of the extract, the dose being ½ — 1 *fl* dr. every 4 hours.

Sen Gupta and Dutta (1949) showed that the Kurchi alkaloids were decomposed by heat and strong alkali Sen Gupta, George and Gupta (1950) studied seven different samples of Indian Senna leaves from the Indian market in order to see if these conform to the specification of B P They suggested inclusion of this important drug in Indian Pharmacopoeia and gave the following specifications for the leaves

(*a*) Water soluble extractives to be not less than 30 per cent (*b*) Ash to be not less than 12 per cent (*c*) Acid insoluble ash not more than 2 per cent (*d*) Stalks not more than 1 per cent and (*e*) other organic matters not more than 1 per cent. The results of analysis of three samples of Senna pods of *Angustofolia Vahl* were also recorded

Gupta, Bose, Ganguly and Chopra (1942) studied the Rideal-Walker coefficient values of certain indigenous essential oils and found that the genera *Ocimum* and especially *basilicum* and the species, *O. O. sanctum* showed definite antiseptic properties against *Bact typhosum*.

Ten varieties of Indian Rhubarb obtained from different localities, such as Sikkim, Assam, Nepal, Dehra Dun, Kashmir, etc were examined pharmacognostically, chemically and pharmacologically by Mukerji (1943 *b*) and Roy, Guha, Bose and Mukerji (1944) and compared with authentic specimen of 'official' rhubarb and their observations were recorded. The evidence pointed to the fact that at least certain varieties of Indian rhubarb might also be recognised in the pharmacopoeias for medicinal use provided they conform to the specifications laid down in the B P. and/or U S.P.

Mukerji and Ghosh (1945) undertook the investigation of Lobelia nicotianaefolia as a possible substitute for *L. inflata* of the B.P. and the results obtained reported.

The action of diastase in liberation of alkaloid from plant tissues was investigated by Gupta and Sen Gupta (1946). The action was more conspicuous in case of seed and bark which contained more of cellulose matter. The aqueous extract obtained after digesting the drug with diastase, contained besides various degradation products, alkaloids almost free from gummy and resinous matters

Sen Gupta, Datta and Banerji (1948) tested various samples of Kalmegh obtained from the market and also of fresh plant by following the method described in the Indian Pharmacopoeial list of 1946 and found that the andrographolide obtained by this method was not pure and a considerable amount of impurities (about 33.6 to 36 per cent) was present in it. Sen Gupta, Banerjee and Chakravarty (1949) attempted to find out a method for the estimation of andrographolide in Andrographis paniculata Nees. The result of analysis of a sample Kalmegh has been recorded.

The colour tests for identification of Aloes barbadensis were applied to Aloe vera Linn. (Ghritakumari) by Sen Gupta and Gupta (1949 *a*) and they concluded that Aloes Vera Linn gave almost all characteristic colour reactions similar to Socotrine Aloes.

Sen Gupta and Das (1948) concluded from analysis of samples of asafoetida procured from the market that none of the samples satisfied the specifications laid down in the official pharmacopoeia. The alcohol soluble extractives were low and the saponification value was rather high

No specification has been laid down for Kaladana in the Indian Pharmacopoeial List of 1946, excepting a test for identity which only indicates the absence of emodin group of compounds in the drugs. Sen Gupta and Gupta (1948) analysed five different samples of Kaladana especially with regard to their resin content, which is supposed to be the active constituent of the drug. They found that Kaladana contained about 14-15 per cent resin of saponification value 181.0.

Rathnasabapathy, Rao, Krishnaswamy and David (1949) studied the toxicity of *Calotropis gigantea* The leaf powder was found to be toxic to canines and non-toxic to ruminants such as calves and goates. It was found to exert a tonic effect on the ruminants. Two substances having the same physiological activity were extracted from the leaves and from the milk juice, the one from the latter being more powerful. Both the active principles produced a sustained rise of blood pressure when given intravenously to anaesthetised dogs due possibly to the direct action on the musculature of the heart and blood vessels. They increased the irritability of the frog's heart and decreased the latent period. The refractory period was shortened after the drug. It was found to contract the uterus *in situ* and isolated, before as well as after ergotoxine.

Swift, Sethi and Sareen (1949) observed that the fresh juice of *Tephrosia purpurea* Var. Pumila or Sarpankha could produce slight transitory hypoglycaemia. The active principle was, however, found to be liable and its activity varied with the season of collection of the plant material.

The pharmacological action of an active constituent isolated from *Daemia extensa* Linn. (Syn. *Pergularia extenta*) was investigated by Gupta, Roy, Ray and Dutta (1950). Daemia and pituitary extract (posterior) appeared to differ in their

site of action on uterus. Daemia appears to have beneficial effect on atonic intestine of paralytic ileus. A state of mild temporary hyperglycaemia seems to result from administration of Daemia. It increased the total acidity of the gastric juice, particularly after a milk feed.

Dutta, Ghosh and Gupta (1950) studied the alkaloidal content of *Holarrhena antidysenterica* Wall (Kurchi) during different parts of the year and of varying ages between 5 and 25 years.

Chopra, Kohli and Handa (1950) found that the bark of Indian Cascara, *Rhamnus virgatus* Roxb. contained only small quantities of oxy-methyl anthraquinones. It was shown to have no purgative action on animals and human volunteers.

VII Snake Venoms

A considerable amount of useful work was done on the Russell's viper and cobra venoms during the period under review Ahuja and Brooks (1948) reported on the mode of action of Russell's viper (Daboia) venom. Their experiments support the view that davoiat venom consists of only one fraction of any pathological importance i e strong blood-coagulant fraction Histological examination of tissues of animals poisoned by davoia venom poisoning, shows that there is extensive deposition of fibrin in capillaries. This deprives the circulating blood of its fibrin content rendering it incoaguable. Such incoaguable blood is itself a coagulant proving that no 'anti-fibrin ferment exists in the venom.

Active principles from the venoms of *Bungarus fasciatus* and *Vipera russellii* were isolated by Ghosh, De and Bhattacharya (1939). They obtained a very concentrated preparation of the active principles of Russell's viper venom by fractional precipitation with sodium sulphate followed by chromatography over aluminium hydroxide C. For the same nitrogen content the activity of the neurotoxin in this sample was about 7.8 times greater than that in the crude venom A very active sample of the neurotoxin of *B. fasciatus* venom free from haemolysin was prepared by fractional precipitation with sodium sulphate followed by chromatography over tungstic acid. For the same nitrogen content the activity of the neurotoxin in this sample was found to be about 5.3 times greater than that in the crude venom. Repeated treatment of a solution of *B fasciatus* venom with ferric hydroxide in the presence of 0.75 per cent of NaCl and at pH 7.2, yielded a sample in which the activity of neurotoxin for the same nitrogen content was about 9 times greater than that in the crude venom. The neurotoxin sample, however, contained some haemolysin.

Ghosh and Kundu (1940) studied the reaction between *Vipera russellii* venom and its antivenene and showed that the neutralisation curve of *Vipera russellii* venom by its antivenene could be represented by an equation of the form:

$$P = \frac{(K + C + TN) - \sqrt{(K + C + TN)^2 - 4TNC}}{2}$$

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lysin which could be restored by treatment with hydrogen sulphide and reduced glutathione.

A very active sample of neurotoxin was separated from the crude cobra (*Naja tripudiana*) venom by Ghosh, De and Chaudhury (1941). The pigeon and mouse units of this fraction were 0 0061 mg and 0.022 respectively. The action of a number of reducing agents on solutions of cobra neurotoxin was tried *in vitro* and it was found that sodium bisulphite, zinc and hydrochloric acid (N/10), ascorbic acid and cysteine could destroy the neurotoxin to a marked extent.

A remarkable increase in the trypsin content of plasma was obtained with cobra venom *in vitro* up to a dose of 0.12 mg per kilo by Iyenger, Dutt and Mukerji (1942) and the value was lowered with increased dosage It was suggested that the venom might be releasing into the blood stream trypsin from the tissues. Iyenger, Sehra and Mukerji (1942 *b*) reported that plasma trypsin was reduced considerably in cases of malignant growth and that Chopra and Chowhan (1935) recommended the administration of cobra venom solution in therapy of cancer The decrease in plasma trypsin brought about by larger doses of the venom was explained as due to the trypsin inhibitor reported to be present in the venom.

Chopra and Chowhan (1940) tried the venom of Indian cobra (*Naja Naja*) in various types of vague and indeterminate painful conditions. In about 70 per cent cases there was marked and considerable relief of pain and in about 30 per cent cases the relief was doubtful or indefinite The pain disappeared slowly but the effect was lasting. No untoward symptoms were produced by the injections.

The effects of neurotoxin, haemolysin and choline esterase isolated from cobra venom on heart, blood pressure and respiration were investigated by Sarkar, Maitra and Ghosh (1942). Purified neurotoxin separated from crude cobra venom had the effect of augmenting the force of the heart beat in perfused toad's heart. With concentrated and repeated small doses there was noticed irregularity with ventricular block which disappeared with the washing of the heart. Neurotoxin also was found to stimulate rabbit's or guineapig's heart with intact circulation. Unlike crude cobra venom it did not cause stoppage of the heart even in higher doses in the toad, the rabbit or the guinea-pig. Neurotoxin has no definite action on blood pressure of rabbits and it paralyses only respiratory movements. In perfused toad's heart haemolysin produced augmentation and depression and irremovable irregularity and ventricular block without causing cardiac failure. Haemolysin in weak doses had no effect on circulation or respiration but in heavy doses caused failure of both. Choline esterase in high concentration slightly stimulated the perfused toad's heart, but had practically no action on the blood pressure and respiration of rabbits.

Taylor (1940) suggested standardisation of different cobra venoms on account of differences found in toxicity of different samples. The assay should be carried out on the basis of the number of 'certain lethal doses' (d c.l) neutralised by a given quantity of serum. A simpler method of calculation is given on the basis

of proportion found to exist between the amount of antivenene required to neutralise 1 d.c.l. and its multiples.

Chopra, Chowhan and Chopra (1942) found that filtration through Seitz filter, though recommended as the best method of sterilisation of snake venom preparations, it markedly reduced the toxicity of Russel viper venom by holding back some of its active principles thereby lowering the therapeutic efficiency of the preparation. Sterilization by sodium merthiolate, phenol or tricresol was suggested as the more suitable method since it could keep all the active principles of the venom intact. Particular care has, however, to be taken with regard to the collection of the material and the preparation of the venom solution.

Ghosh and Chatterjee (1948) reported an active principle from cobra venom which inhibited the cytochrome oxidase system in the tissue cells. Chatterjee (1949) found the purified product to be 16 times more active than the crude venom on the weight basis. The inhibitor was fairly stable at moderately high temperature, wide range of pH (1.0 to 9.6) and was unaffected by exposure to ultra-violet rays lasting for a couple of hours.

VIII. Anaesthetics

Very little work was done on this subject. Sircar (1947) made a statistical analysis of his 1,000 clinical administrations with cyclopropane and described its pharmacological actions. He was of the opinion that cyclopropane could be a pleasant non-toxic powerful and controllable anaesthetic agent for almost every type of case.

Bose and Mukerji (1944) made a comparative study of anaesthetics and toxic action of trichlorethylene, chloroform and ether on mice, guineapigs and rabbits. Trichlorethyline showed certain characteristics common to both chloroform and ether, but muscular relaxation was almost as imperfect as with nitrous oxide. It was less toxic than chloroform but ether was safer. The anaesthetic dose was slightly greater than that of chloroform but was much less than that of ether. It had some favourable features such as, rapidity of action, analgesic effect and relative absence of early fatal accidents. In heavy doses it had toxic effect on kidneys and lungs.

A simple and inexpensive method of intra-tracheal anaesthesia had been described by Ganguli (1939).

Bagchi, Ganguli, Mukerjee and Banerjee (1939) gave an account of a new method for determination of small quantities of cocaine in presence of novocaine.

IX. Metals

Interesting work of far-reaching importance was done during the inter-regnum reviewed herein on metals like lead, arsenic, copper, iron, etc. The following

abstract do not give exhaustive accounts of all the work done on these metals in their different aspects, but only in so far as they have a bearing on pharmacology.

Investigations were undertaken on the presence of lead in the different tissues of our body and its effects on our system. Bagchi, Ganguli and Sirdar (1939) have found lead to be a constituent of almost all the human tissues and individual variations are suggested to be due to dietary habits Bone, teeth and hairs are good sources of lead in the human body. The maximum amount is found in hair, especially the black hair of Indian women. The colour of hair appears to depend on its lead content The skin, in spite of the fact that it is histologically closely related to hair and contains numerous hairs and hair follicles, is very poor in lead. The ovary is the only organ which has been found free from lead and thus it differs from testis which contains quite an appreciable amount. The foetal tissues do not show *any affinity for lead although it is believed otherwise* In case of abnormal exposure to lead, the liver, stomach, kidney and lungs show well-marked increase in their lead content and thus indicate the route through which lead gets into the system.

Bagchi, Ganguli and Sirdar (1940 *a*) have also found that hair contains large amount of lead and in case of abnormal exposure it may retain as much as 508 mg per kilo. Large quantities of lead in urine and faeces of persons having a very high lead content of the hair, seems to indicate that lead is absorbed into the general circulation and then eliminated through the hair Possibly lead is present in the hair in combination with phosphorus

The presence of lead in the various foodstuffs which are commonly used has been determined by Bagchi, Ganguli and Sirdar (1940 *b*) and the result of their investigation shows the presence of lead in appreciable amount in several of our food stuffs of animal and vegetable origin

Bagchi (1941) also investigated the problem of lead poisoning of industrial or occupational origin

The presence of other poisonous metals in human tissues and their significance and medico-legal bearing have been reported by Bagchi (1941). He also worked out the lead content of foetal tissues and explored the question of lead poisoning in children (1942).

Bagchi and Ganguly (1941 *c*) assayed the arsenic content of some common articles of Indian diet. Bagchi (1941) traced the source of the arsenic component of all human tissues to the food ingested

The copper content of a number of cereals, pulses and a few other Indian foodstuffs was assessed by Chowdhury and Basu (1939). The amount of copper in different food-stuffs which was rather high, would in consequence produce no serious copper deficiency in any adult mixed diet. But the low percentage of copper

in cow's milk, suggested the desirability of adding a trace of copper salt in order to enrich the infant dietary.

Datta (1941) also observed that the copper content of some of the Indian foodstuffs ranged from 0.18 parts per million in milk to 12.5 parts per million in cereals. 94 to 96 per cent of the copper added as copper sulphate to wheat or to milk was excreted in faeces. The presence of a very slight but a constant quantity of copper in the urine indicated poor absorption of the metal. The copper that enters food from vessels due to contamination is probably metabolised in a manner very similar to that of added copper. There is, however, indication of a slightly higher excretion in urine and increased retention in the body. Groups of rats which were kept on a diet of Chapatti with supplements of milk to which was added 6 mg and 9 mg of copper as copper sulphate appeared less active, with hairs slightly pigmented and showed growth rate lower than those of the controls.

Aykroyd and Krishnan (1939) gave supplement of 1 gramme of calcium lactate to South Indian day school children for a period of about 11 weeks and noted significantly greater increase of height and weight than children not receiving calcium. In a similar group, receiving skimmed milk, height and weight increments were also significantly in excess of those recorded in the control group Skimmed milk group showed more improvement in general condition than the calcium lactate group.

Chatterjee and Ganguly (1950) estimated the amount of copper in human urine and faeces of people of different nationalities; that copper possessed a definite bactericidal action against *V cholerae*, *B dysenterias* (shiga), *B. typhosus* and *B. coli* of which *V cholerae* was shown by Bose and Chakraborty (1948).

Some of the iron present in fish appears to be present as an iron-protein complex, which can be easily hydrolysed by pepsin and trypsin (Saha and Guha, 1940). During egg formation the available iron content of the muscle of the fish is greatly decreased, while iron is found concentrated in the roe.

Although most of the plant foodstuffs are fairly good sources of total iron, the ionisable (Assimilable) iron content found by Roy, Pal and Guha (1939) is only a small fraction of the total iron in all cases, except in potato of which the ionisable and total iron contents are comparable. Investigation of the cooked diets in adult students, hostels in Calcutta revealed appreciable deficiency in the ionisable iron intake.

Pal (1939) has reported that both the total and ionisable iron present in cow's milk are comparable. Almost all the iron present in mother's milk is in available form.

X. EPIDEMIC DROPSY

Epidemic dropsy is an evil dispensation for some of the provinces in India. This problem was tackled by some of the workers, mainly of the All-India

Institute of Hygiene and Public Health and of the Calcutta School of Tropical Medicine Their investigations primarily centred round the solution of the cause of the disease. Though the etiology still remains unveiled yet the pendulum swings in favour of the mustard oil theory. Its adulteration with argemone oil is supposed to be the cause of the mischief and most of the work has been concentrated round this adulterant.

Pasricha, Lal and Banerji (1940) studied the effect of oral administration of argemone oil to laboratory animals and found it to be more toxic than mustard oil, olive oil or liquid paraffin When argemone oil is heated to a stage at which it fumes well (at 240°C for 15 minutes) the toxicity of the oil is not much more than that of certain bland oils, such as, olive oil or liquid paraffin. The heated oil still gives a positive nitric acid test. Heating the oil at 100° or 150°C has no appreciable effect in its toxicity to animals Argemone oil when given by mouth to guinea pigs or mice causes extensive degenerative changes in the liver and the kidneys.

A quantitative chemical test has been developed by Lal, Mukerji, Das Gupta and Chatterji (1940) and gauging curves have been presented by means of which the content of the reacting substance of the oil and roughly the percentage of the argemone oil can be determined in samples of mustard oil. It has been shown that the content of the reacting-substance of the toxic mustard oil diminishes under the action of light (diffused and direct) particularly in presence of air Air alone does not cause this reduction. It has been recommended that sale of mustard oil containing 0.5 per cent or more of argemone oil, for human consumption should not be permitted. For purposes of quantitative test it is necessary to submit fresh samples of oil in dark-coloured phials which should be filled to the brim to exclude air.

The fraction obtained from argemone oil by the process of saponification and extraction with hydrochloric acid does not prove toxic to man although it reacts specifically to chemical tests and induces essential histological changes in white rats. The residue of argemone oil left after extraction of the fraction with the hydrochloric acid is not toxic to man It has been further observed that adequate exposure of argemone oil to light renders it non-toxic These observations suggest that the treatment so far employed, isolate only a portion of the toxic radicle and neither of the split products are toxic. Re-combination of the split products into the original toxic molecule has not been effected by the methods so far employed (Lal, Das Gupta, Agarwal and Adak, 1941).

Signs and symptoms of epidemic dropsy can be produced in man by feeding with oil expressed from seeds of *Argemone maxicana* (Chopra, Pasricha, Goyle, Lal and Sen, 1939).

Feeding experiments have been carried out by Lal, Chatterji, Agarwala and Das Gupta (1941) in a number of animals, namely rats, cats, guineapigs and

pigs with a view to find out suitable biological tests for the detection of specific toxicity in respect of epidemic dropsy in samples of suspected mustard oil. Addition of pure argemone oil or 10 per cent of argemone oil in mustard oil or of epidemiologically incriminated and experimentally proved toxic oil to the standard diet causes great depression of the growth curve Histological changes in the skin of rats so fed resemble those found in the skin of patients of epidemic dropsy. Capillaries are dilated and there is perivascular infiltration of endothelial cells. Cats fed on toxic oil show also similar changes except that in the later stage of the experiment the vascular walls are collapsed and do not exhibit the characteristic young endothelial cells.

Methods of isolation of a crystalline substance and a free bash responsible for the reactivity of the argemone oil (or mustard oil epidemiologically associated with epidemic dropsy) have been described by Mukerjee, Lal and Mathur (1941). Both of them contain a radicle recognisable in the differential tests of the toxic oil. Argemone oil, which has been exposed to light for a sufficiently long period to greatly reduce its reactivity to the differential test, yields a very poor crop of the reacting substance or the free base It is presumed that the isolable products are only the components of a complex toxic substance in the oil

Lal, Das Gupta, Agarwala and Adak (1941), applied the biological test to modified argemone oil and its derivatives. The histological findings indicate that like the argemone oil, the crystalline substance and to a certain extent the free base exert poisonous effect on rats when added to the food, but given subcutaneously, the crystalline substance does not manifest such result; argemone oil after removal of the free base or modification through exposure to light with loss of reactivity to the chemical and physical tests become biologically inactive As may be expected addition of the crystalline substance in the former case brings back the poisonous properties Mustard oil by itself does not cause any poisonous effect

A new simple test for Argemone Oil has been devised by Chakravarti, Chaudhuri and Chakravarti (1950). It is extremely sensitive, being found positive in a concentration as low as 0 1 per cent

XI DRUG ADDICTION

The Drug Addiction is a menace to the people of India from the social, moral and economic point of view The enquiry into this problem was started by Sir Ram Nath Chopra in 1926 and since then an enormous amount of important work has been published by him and his collaborators from the Calcutta School of Tropical Medicine. The subject is vast and investigations in its varied aspects is a difficult and stupendous task. Sir Ram Nath studied the effects produced by these drugs and also noted the incidence of addiction in different parts of this country.

The use of hemp drugs in India was discussed by Chopra (1940). Withdrawal syndrome in opium addicts and the rationale of treatment with lecithin and glucose was discussed by Chopra and Chopra (1940 a).

Sir Ram Nath studied the depressing and painful condition of these individuals and in order to ameliorate these fearful abstinence symptoms he and his associates concentrated their attention on the treatment of withdrawal syndrome during this period The effects of treatment with glucose (Chopra and Ganguly, 1939) and with lecithin and glucose (Chopra and Chopra, 1940) were noted. Biochemical and biophysical investigations into the blood of opium addicts evinced that withdrawal of opium caused an increase in the total proteins in the blood sera, thereby indicating a loss of fluid from the body.

Chopra, Mukherjee and Chopra (1935) observed an increase of euglobulin in the blood of addicts which probably meant an ultimate drainage of phosphate from the nerve cells and as such the lecithin treatment was suggested In majority of cases lecithin decreased the intensity of withdrawal symptoms and shortened their duration Still, abstinence symptoms were severe in some subjects The ultimate effect of treatment with glucose seems to be the restoration of water balance. Any drug that confers fluid retaining power to the blood is expected to have good effect and glucose is known to possess this water retention property Glucose, therefore, not only stocks glycogen in the liver to enable it to cope with the unusual strain on this organ during the process of elimination of morphine but also helps the retention of water in the blood Lecithin, therefore, tones up the nerve of addicts and glucose helps to restore the disturbed water-balance Treatment of opium addiction with lecithin and glucose brings the total proteins to their pre withdrawal level It appears that the central nervous system and the digestive system bear the brunt of this addiction and symptoms produced during the abstinence period are greatly modified and ameliorated by this treatment. The results of the treatment proved to be very encouraging and better than other forms of treatment tried by them It is suitable for mass treatment (Chopra and Chopra, 1949) and has been used in Assam in a series of over 12,000 addicts, with very good results and at a reasonable cost Guha Roy (1939) also reported encouraging results with this treatment

Chopra, Chopra and Chopra (1942 *a*) suggested the possibility of Eucodal habit, which is no less serious than that from morphine or heroine.

Urinary excretion of morphine in opium addicts with and without lecithin-glucose treatment has been investigated by Chopra, Chopra and Roy (1941) They have observed that the time taken to render urine morphine-free appears to be shorter in the case of addicts treated with lecithine and glocose than in the untreated cases. The rate of excretion of morphine bears no relation either to the daily dose of opium or to the duration of addiction. The morphine content of a sample of urine is not appreciably affected on keeping it for several days

The relation of *Cannabis sativa* to mental diseases and crime in India has been studied by Chopra, Chopra and Chopra (1942 *b*).

XII. DRUG ASSAY

A fair amount of useful work has been done especially at the Central Drugs Laboratory, Calcutta, in connection with standardization of different preparations. In some cases new methods have been devised and in others original methods have been modified to suit the requirements of this country.

David and Krishnaswami (1939) have suggested the use of dogs in place of cats for bio-assay of digitalis without any sacrifice of accuracy of result. The dogs are cheaper, easier to get and more convenient to handle than cats. The dogs usually give a lower figure as compared to the standard but the error is within experimental limits and any way not more than 10 per cent. Generally speaking the m.l d. for dog is slightly higher per kilogram weight. The individual variation in m.l d when a number of dogs are used is not more than what is found with cats.

A simple method of assay of digitalis in India by using guinea-pigs has been described by Bose and Mukerji (1942). The m l.d. of International standard digitalis tincture in a series of 30 guinea-pigs has been found to be 9.07 c.c. ± 0.381. The workers think that this method gives very dependable results.

A modified method for determination of 'Prothrombin time' has been described by Iyengar, Sehra and Mukherji (1942 *a*). The stability in thromboplastic potency of dilute solutions of Russell's viper venom, both in water and in 0.025 M calcium chloride solution has been studied in detail. The results obtained indicate that the solutions when kept under toluene at a temperature of about 5°C are quite stable and hence standardised stock solutions can be used for routine prothrombin determinations. A slightly modified method for the determination of prothrombin has been suggested The prothrombin time is slightly speeded up by the revised method

Iyengar (1943) has detailed a method of assay suitable for determining the activity of papain The rate of deterioration of samples of papain of various grades of purity, when kept at the temperature of about 25°C for periods of 3 to 6 months has been studied.

An account of an *in vitro* test for estimation of the anti-haemolytic titre of cobra antivenene has been given by Ahuja and Brooks (1944). The anti-haemolytic potency of the immune serum corresponds closely with its anti-neurotoxic titre as determined by the *in vitro* test on pigeons.

A sensitive method for quantitative estimation of small quantities of common trihalogen volatile anaesthetics in blood and tissues of animals has been described by Kulkarni (1944).

Bose, Iyenger and Mukerji (1945) have made some observations on the assay of urea stibamine. The LD_{50} figure for the selected batch of urea stibamine has been found to be 215 mg/kg for white mice of 18 gm to 20 g weight. Solutions of urea stibamine are much more stable than solutions of organic arsenic compounds and it has not been considered necessary in assaying urea stibamine to observe the elaborate precautions in the preparation and use of solutions, which *must be followed in the case of organic arsenicals. The relationship* between the total antimony content of urea stibamine and its toxicity is not known as yet. As the chemical composition of urea stibamine is variable and as it is not yet definitely known which fraction or fractions are therapeutically active, the authors suggest that the assay of unknown preparations of this drug should, for the present, be carried out by the biological method.

Assay of anthracene purgatives (Rhubarb) by the estimation of the content of *hydroxymethylanthraquinones has been suggested by Ghosh, Gupta and Kahali* (1945). According to these workers the hydroxymethylanthraquinone content of rhubarb is a correct indication of the purgative potency. The purgative effect of the different rhubarb samples has been found in cats to be related to their hydroxymethylanthraquinone content Samples of rhubarb containing about 2 per cent of hydroxymethylanthraquinone, even though they contain alcoholic extractive far below the minimum B P. standard, have been found to be pharmacologically as effective as any B.P. standard rhubarb

Great difficulties are encountered in this country to study the anti-malarial action of drugs in laboratory animals. Cannaries and Java Sparrows on which the gametocidal activity of drugs is generally tested are sometimes rarely available. Nature pigeons, infected naturally with Haemoproteus columbae, are however found in plenty and have been used to develop a technique for gametocidal assays of certain quinoline and acridine compounds with and without the substitution of *sulphonamide group in the benzene ring* (Bose and Rakshit, 1944). The results of study indicate that the method of assaying in pigeons would probably be useful in coming to definite conclusions regarding the gametocidal activity of different synthetic drugs

A satisfactory method of bio-assay of adrenaline on guinea-pigs has been developed by Bose and Ghosh (1945 *c*). *Chowdhury, Roy and Mukherji* (1948) have pursued the assay of adrenaline by the method developed by Kundsen *et al* calculating the results from the chart and minograph supplied. Spinal cats, however, have been used instead of anaesthetised, atropinised dogs. Results of nine assays reported compare favourably with those of Kundsen *et al.*

Standardisation of liver extracts is a matter of great concern in our country. This is all the more necessary from the fact that the crude whole liver extracts *are better than highly purified liver extracts* in the treatment of tropical macrocytic anaemia. No standards have up till now been laid or accepted on the basis of

which the preparations of liver extracts in this country can be standardised. This state of affairs naturally brings difficulties for the manufacturing assayists and clinicians (Bose, 1949 *d*, 1950). In the absence of clinical standardisation, attempts have been made to standardise liver extracts using laboratory animals. Two methods have been developed on the basis of reticulocyte response on normal guinea-pigs put on a standard diet (Bose, 1949 *e*, *f*), and the other on spleen-ectomised rabbits (Bose, 1949 *g*) While potent liver extracts can be qualitatively as well as in quantity assayed by both the methods, in the guinea-pig method the animals became refractory after the first injection on liver extract. Spleen-ectomised rabbits do not behave in such a manner.

Dutta and Macintosh (1949) gave an account of the procedure of the assay of curarising drugs by means of the head-drop reaction in the rabbit

By using the experimental procedure used by Ipsen (Ipsen, J, *Bull Health Org*, 7, 785, 1938) Hazra, Lahiri and Sokhey (1945-46) standardised Haffkine Institute polyvalent anti-snake-venom serum against the venoms of the four common Indian snakes namely cobra, common krait, Russel's viper and saw-scaled viper.

Dutta, Mukherji and Chandrasekhar (1946) explored the possibility of using common Indian domestic pigeons (Columba livia intermedia) in toxicity determination of organic antimony compounds e g urea stibamine Statistical comparison with parallel figures obtained with white mice showed a close agreement with pigeons and the accuracy of the assay results obtained was also of a high order

Two varieties of tadpoles (*Rana tigrina* and *Bufo melanostictus*), have been used by Dutta and Mukerji (1942 *a*) in their work on bio-assay of thyroxin and similar preparations The Rana variety, in the opinion of the authors, are more suitable for the work. The results obtained from assay of different preparations point to the fact that biological activity is probably more related to total iodine in thyroid preparations, rather than to thyroxineiodine.

Chowdhury, Dutt and Mukherji (1944) made some observations on the estimation of diuresis inhibiting activity of posterior pituitary extract.

Investigation for detection of pyrogens in fluid used for intravenous injection by biological methods was undertaken by Bose and Ahuja (1944). Normal temperature variation in 100 rabbits was worked out. Two strains of pyrogenic organisms were isolated from a public reservoir and their character studied. A comparison of the hyperpyrexia and leucopenia tests as carried out in rabbits using suspensions of these two organisms showed that only the former was a fairly satisfactory method of pyrogen estimation.

Iyengar, Bose and Mukerji (1945) have attempted some modifications on the assay of insulin in India by the 'Rabbit method'. The fasting blood sugar of rabbits in India has been determined in 40 rabbits and the average value has been found

to be 118 8 mg per 100 cc. with a standard deviation of 13 4. The response of these rabbits to graded doses of insulin has been determined The response to the same dose of insulin, as measured by the British method of pooling equal quantities of hourly samples of blood and by the Toronto method of pooling from three bleedings at intervals of 1½, 3 and 5 hours has also been determined.

Biswas, Mukerji and Iyengar (1947) in connection with their work on the assay of Globulin-insulin preparations have made a comparative study of the hypoglycaemic effect at different intervals with one dose of globulin insulin, given at one time and the same dose of regular insulin in two portions five hours apart. The average hypoglycaemic effect of globulin insulin in seven hours is approximately equal to that of regular insulin during the same period provided the latter is given in two doses of five hours apart

As a preliminary step to the determination of potency of lactogenic hormone, the establishment of the 'crop-gland stimulation' technique of Indian pigeons with international standard prolactin, has been attempted by Bose and Mukerji (1947) A dose-response curve showing the relation between various doses of the International Standard of 'prolactin' and the percentage increase in the weight of the crop-glands of pigeons has been presented for the general assay of the lactogenic hormone in India

Chowdhuri and Mukerji (1947) worked out the antidiuretic potencies of posterior pituitary extracts by a modified method based on the assay procedures suggested by Burn and Gibbs and prepared a standard curve for reference Oxytocic and anti-diuretic potencies of different samples were compared and found to run parallel.

Lal (1950) estimated six essential amino acids (tryptophen, lysine, histidine, phenylalanine, arginine and threonine etc in gram and ragi by microbiological technique. All the amino acids estimated were found to be present in useful amounts but threonine was absent.

The suitability of common Indian domestic pigeon (Columba livia intermedia) as test object in evaluating anti-anaemic factor in liver preparations was investigated by Ghosh and Mukerji (1950). Quantitative relationship between the dose and the resulting reticulocyte response was observed. Assay of liver extracts by the reticulocyte response of normal guineapigs was made. The method would be useful in differentiating the relative efficacy of several products.

Laskar (1944), Mukerji and Dutta (1945) suggested some modifications in existing procedure for the assay of entero-vioform and alkaloids in Kurchi Bismuth Iodide respectively. Colorimetric method of determination of Pteroyl Glutamic Acid and related compounds in liver extract concentrate has been described by Ganguly (1950). A method of estimation of Procaine in solution was described by Basu (1949). Bose (1949 *g*) recorded some preliminary observations on the assay of liver extracts on spleenectomised rabbits.

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A method of assay based on the antagonistic effect of anti-histaminic drug, as Benadryl etc on histamine-containing tissue extracts such as liver extracts, etc has been presented by Chowhan and Mukerji (1950). This method estimates fairly accurately minute traces of histamine in such tissue quantitatively.

XIII. Toxic Effects

An appreciable amount of useful work has been done not only on the toxic manifestations of some products that have been introduced into the market but also of some cyanogenetic substances that are present in vegetables and other foodstuffs. Reports on the effects of some food colours have also been published.

Konar, Roy and De (1939) described a case of naphthalene poisoning Symptoms of poisoning with sulphonamide group of drugs have been recorded by De and Konar (1940), Rajam and Vasudeva Rao (1941), Man Singh (1942) and Berry (1944) De and Konar reported cyanosis and haemolytic condition, haematuria and methaemoglobinuria in sulphanilamide therapy, while Rajam and Vasudeva Rao discussed the cutaneous reactions and the blood dyscrasias on the use of the same drug Man Singh described the toxic effects of sulpha-pyridine on the liver. Hepatic cells showed cloudy swelling and certain amount of degenerative changes amounting to necrosis. All other organs were practically normal Berry noted angioneurotic oedema produced by sulphathiazole

Phenomenon of auto-haemagglutination in man after sulphapyridine treatment in a case of respiratory infection was described by Parekh (1943). The agglutination has been found to be most marked in cold and feeble or inactive at 37°C The process of auto-agglutination is not constant and is reversible. Further, it has been shown that agglutinins could be absorbed Sulphapyridine anuria has been noted by Waterston and Doherty (1943) Cardiac irregularities caused by sulphapyridine and sulphathiazole have been recorded by Chand and Chand (1947).

Rajam and Vasudeva Rao (1939) discussed the toxic reactions observed with mapharside after treating 1,000 cases of syphilis in all stages with it

While administering sulphonamide group of drugs Niblock (1941) did not find it necessary to put the patients on any special dietary restrictions Sulphates may be given in therapeutic doses provided proper care is exercised The drug is to be stopped in case of occurrence of cyanosis. Bardhan (1943 *b*) did not observe sulphaemoglobinaemia after administration of sulphur through drugs and foods in course of sulphonamide therapy. No cyanosis was noticed by him

Respiratory distress following administration of barium meal has been reported by Chopra and Chakrabarty (1940).

Bagchi (1941 *a*) investigated the incidence of lead poisoning among Hindu women and children and suggested red vermilion, which contained red lead mixed

with red synthetic dye, to be the source of chronic lead poisoning amongst Hindu women.

A peculiar neurological sequel to administration of 4: 4′—diamidinodiphenylethylene (M & B 744) in kala azar cases has been described by Napier and Sen Gupta (1942). The patient developed anaesthesia to light touch over the trigeminal area with preservation of sensation of pain, temperature and pressure. The lesion, according to the workers, is apparently in the pons involving the principal sinsory nucleus of the trigeminal nerve and is possibly a toxic degenerative condition. Neither the lesion is progressive nor its symptoms produce much physical discomfort. The condition has also been found to improve in time. Sen Gupta (1943) on further observation met with similar neuropathic sequel. Diamidino-stilbene has been found to be the toxic factor responsible and the selective toxic action is probably due to the ethylene component of this compound. In cases having troublesome paraesthetic symptoms, all non-surgical therapeutic measures have been found to be useless, except injections of the solution of cobra venom (1· 100,000) in gradually increasing doses, which has been noted to produce some degree of subjective improvement.

Guha, Dutta and Mukerji (1943) have tested more than a hundred samples of urea stibamine (Brahmachari). The chemical assays for antimony content and toxicity tests on white mice have proved the antimony content of urea stibamine to be fairly constant and it has been found between 39 and 42 per cent. In majority of experiments a toxicity figure varying between 200 and 225 mg per kg has been recorded. Bose, Ghosh, Mitra and Dutta (1946) have also studied the toxicity of a large number of samples of urea stibamine and other pentavalent organic antimony derivatives used for the treatment of kala-azar in relation to their antimony contents. It has been found that the toxicity of urea stibamine does not depend solely upon the total antimony content but is influenced by the presence of antimonious acid. Urea stibamine, if carefully prepared, can be made to satisfy much higher toxicity limits than those of 200 to 225 mg per kg as accepted at the present time The toxicity of the diethylamine and tri-iso-propylamine salts of p-aminophenylstibinic acid depends to a considerable extent on the purity of these amines used.

Working on the toxic effects of Teora (Khesari, *Lathyrus sativus*) Jacoby (1947) concludes that this is due to fungi growing on defectively stored wet teora grains.

A marked toxic effect on the elimentary system of the frog with thiamine chloride, a property not shared by nicotinic acid, has been observed by Zaidi (1947). A powerful toxic action on the heart of frog has also been noticed in large doses.

An unusual phenomenon of sudden and complete relaxation of all the muscles, in strychnine convulsions, so as to permit free movements of all joints has been described by Shirazi (1947).

Govinda Rao (1947) has recorded a case of maniacal excitement following large doses of atebrin The toxic manifestations of mepacrine have been described by Chakravarti and Misra (1946). Two cases of mental symptoms after quinacrine therapy for malaria have been reported by Berry (1945). After parenteral administration of quinacrine in malarial fevers only two cases have shown major mental symptoms out of a total of 6497 cases treated by Basu Mullick and Gupta (1947 *a*). The relapse rate has been found to be 11.6 per cent. Heilig and Visweswar (1944) studied the influence of intravenous injections of quinine on the myocardium 40 grains of quinine bihydrochloride injected in five divided doses, caused myocardial damage of more or less severe degree in a high percentage of malaria cases which clinically and radiologically showed no signs of serious pathological heart condition, at the onset of the course. A normal electrocardiogram and even a physiological reaction to an exertion test were no safeguards against such toxic bye-effects. Therefore, they warned this method of treatment should be used only if strictly indicated.

Draper (1947) assessed the side effects of pethidine by taking the drug himself in dosage of 50 mg ampoules every 4 hours for 8 days Total dose taken was 1,600 mg He did not find the drug to be constipating and thus differing from morphia. Nor did it produce on him any drying up of salivary secretion, an action different from atropine. There was a decided euphoria but not of so pronounced a character as to cause a rapid addiction. In him its unpleasant effects far outweighed the sense of euphoria. His digestive system was decidedly upset with loss of appetite and a coated tongue. Still worse were the unpleasant dreams.

Calcium administered either as a salt or in combined form as the natural foodstuffs, exerted a mitigating influence on fluorine poisoning in rats. At the level of dosage used, the toxic effect of fluorine was inversely proportional to calcium intake (Ranganathan 1941). He (1944) also observed that different calcium salts were roughly equally potent in mitigating the toxic effects of fluorine in experimental animals (rats). Magnesium salts too conferred a similar protection, though to a somewhat smaller degree. Vitamin C and D had no effect in fluorine poisoning in rats.

Inclusion of sufficient amount of milk powder (Klim) and bone powder in diets of experimental rats afforded remarkable protection against fluorine poisoning. The beneficial effect was possibly due to calcium and phosphorus content in organic combination (Pillai, Rajagopalan and De 1944). The findings of Ranganathan (1941) also pointed to the same conclusion.

Majumdar and Ray (1946) have concluded from their experiments on hill bulls that even when a diet contains adequate amounts of calcium and phosphorus the relief afforded in fluoresis is only temporary and is useless to save a victim from the effects of prolonged high intake of fluorine. They also have noted that the administration of aluminium phosphate prevents the formation of exostoses in

bulls receiving fluorine in their diets. Venkataraman and Krishnaswamy (1949), have confirmed the ameliorating effect of aluminium salts in fluorosis induced in albino rats and have shown that the skeletal storage of fluorine is appreciably reduced by the presence of aluminium in the diet.

Bagchi and Ganguli (1942) have identified the presence of a cyanogenetic glucoside in bamboo shoots (*Bambusa arundinacea* Willd) and have further reported on the toxicity of these young shoots of common bamboo (1943 *a*). They have demonstrated the liberation of hydrocyanic acid from the cyanogenetic glucoside present in young bamboos. The complete hydrolysis of the glucoside by its specific enzyme and the production of maximum amount of hydrocyanic acid are brought about when the bamboo shoot is made into a pulp and soaked in plain water for about two hours. Presence of acids or alkalies prevents the enzymic hydrolysis of such glucosides.

The toxicology of the Linseed plant (*Linum usitatissimum* Linn) has been studied by Bagchi and Ganguli (1939). Cattle poisoning by linseed flowers is fairly common in Bengal and Bihar. The hydrocyanic acid content of the various parts of the linseed plant has been worked out by these workers, the linseed flowers with immature seeds producing the maximum amount. A strong solution of sodium carbonate has been suggested by the workers as an antidote in linseed flower poisoning. As cyanogenetic compounds are found in plenty in the vegetable kingdom and notably in grasses, the authors have suggested the possibility of poisoning by hydrocyanic acid should always be thought of in medico-legal investigations in obscure cases of cattle poisoning. The same authors (1941 *a*) have also studied cattle poisoning by *Sorghum vulgare*, known as 'jowar' or 'junuri'. The poisonous principle is hydrocyanic acid liberated from the cyanogenetic or cyanophoric glucoside known as Dhurrin ($C_{14}H_{17}O_7N$) or parahydroxymandelo-nitrile glucoside present. It is extracted from the fresh roots, stems or leaves by water for 2-4 hours at room temperature. Mineral acids or alkalies in concentration higher than N/10 retard the process of hydrolysis. The detection of the amount present in stomach-content is of medico-legal importance, as it will give an idea whether the death is due to accidental poisoning by sorgham or any other HCN containing grass or due to administration of cyanides by professional cattle-poisoners. A heavy dose of alkali has been found to be an effective antidote.

The toxicology of the fruits of *Zantboxylum acanthopodium* Dc has been studied by Bagchi and Ganguli (1943 *b*). They have found these fruits to contain a resinous substance which produces an intense tingling sensation in the mouth. The irritant principle present in the resin and responsible for the characteristic sensation is an amide, possibly fagaramide.

Bagchi and Ganguli (1943 *c*) have utilised the higher dichromate reducing titre with blood of drunken persons to determine the amount of alcohol consumed. The results not only indicate the amount of alcohol but other volatile oxidisable subs-

tances present in the blood, as for instance, ketone bodies and certain drugs.

Bagchi (1941 *b*) has dealt with the question of the indigenous poisons in crime and difficulties associated in their detection.

The pharmacological and toxicological properties of two food colours, one of coal-tar origin and another of vegetable origin were investigated by Mukerji, Dutta and Mukerji (1943). Chronic feeding experiments on white rats extending for 2-4 months with the dyes in different concentrations were carried out. Post-mortem examination of some organs were made and histopathological changes were recorded

Gupta and Chatterjee (1945-46) have investigated the toxicity of a vegetable dye ('Kamala' powder or *Mallotus phillippinensis*) in a vegetable oil (Dalda) by oral administration in white rats extending over a period of 6 months. The changes in the weight of rats under the dye and of those under the control experiment, according to the authors, appear to be of the same order in all the different groups of experiments and are, therefore, more due to the rejection of the diet, which may arise either from the satiety caused by the oil which is not a natural food for rats or due to some factors other than the toxicity of the dye There has not been any histological changes in the structures of the liver, kidney and suprarenals of rats examined at different intervals, which indicate the absence of toxicity of the drug even when given in fairly high concentration.

p-carbamide-phenyl-arsenious acid has been prepared and tested by Bose, Bose and Ghosh (1950). It has been found to be a toxic drug. The burnt of attack is probably taken by the liver and kidneys.

Konar and Das (1950) have found picrotoxin to be an effective antidote in cases of barbiturate poisoning

Bose, Ghosh, Mitra and Dutta (1946) have studied the toxicity of some organic antimonial drugs used for the treatment of kala-azar.

XIV. Miscellaneous Work

Basir (1940) studied the effect of the autonomic nerves on the backward flow of the perfusing fluid in the spleen of dog. The backward flow for the arterial canula began in the dog's spleen when the pressure of the perfusate was increased to 85 mm to 95 mm of mercury on the venous side. When the perfusate contained acecholine there was an increase (three times) in the backward flow of the arterial canula and also there was an increase in the volume of the spleen, presumably due to relaxation of the plain muscle. When it contained adrenaline a decrease or even a complete cessation of the backward flow of the perfusate from the arterial canula was observed. Decrease in the volume of spleen was possibly due to contraction of its plain muscles On stimulation of vagus and splanchnic a decrease in the backward flow of the perfusate from the arterial canula was noticed on both occasions.

In dog stimulation of the vagus caused a fall in the volume of spleen, possibly due to contraction of its plain muscle

The inhibitory agents of uterine motility were investigated by Krishnan (1940). The effects of antuitrin S, antuitrin, antuitrin G (P D & Co), thyroxine, adrenalin, insulin and vitamin E on *uterine motility were studied in vitro and in vivo after* a series of injections in normal and ovariectomised guinea-pigs and normal cats and rats, pregnant and non-pregnant The results were negative except in the case of adrenalin which had an inhibitory effect on the pregnant as well as on non-pregnant uterus of rat and guinea-pig. Antuitrin and antuitrin G (P.D. & Co) were found to be excitatory. Insulin intensified the pituitrin reaction of the pregnant and non-pregnant uterus of guinea-pig Vitamin E had no direct influence on the uterine motility but promoted the growth of luteal tissue. Chemicals like thymol, acetone, ether and alcohol all had inhibitory effects on uterine motility. Of these thymol and acetone had the most pronounced influence Bile acids were found to *have a negligible effect.*

Sinha (1942 *a*) studied the behaviour of pulmonary vessels in the isolated perfused rat lungs. Electrical stimulation of stellate ganglion or injection of adre*nalin caused vaso-constriction which was, however, prevented by previous injection* of ergotamine. Electrical stimulation of vagi had very little vaso-constrictor effect. Injection of acetyl-choline also produced vaso-constriction which was again counteracted by previous injection of atropine. The same worker (1942 *b*) also noted that injection of ergotamine or eserine also caused vaso-constriction in guineapig lungs

The interaction between ionisable Drugs and Electrical stimulation as indicated by inhibition of unstriated muscle has been studied by Singh (1942). Inhibition is well marked in guinea-pig uterus. Three types of inhibition — chemical, electrical and physico-chemical are known. The last is due to increase in osmotic pressure within the muscle fibres. Just as the contraction produced by electric current is antagonistic to that produced by potassium ions, so also are the inhibitions produced by these agencies. Increase in osmotic pressure produces most of the phenomena produced by electric current. Alternating current produces two kinds of contraction *as well as inhibition. Plain muscle exhibits two kinds of tone,* one due to slow relaxation and the other to the contraction produced by chloride ions The action of sodium and chloride ions in the saline is antagonistic. There are two kinds of spontaneous contractions, one due to alternate contractions and the other to alternate relaxation.

Dutta (1949 *a*) has shown that atropine, pethidine, procaine and quinine have the following pharmacological properties common to them· (*a*) When injected into the fluid perfusing the superior cervical ganglion they depress the contraction of the nictitating membrane in response to preganglionic stimulation. (*b*) They augment the contractions of the isolated rat diaphragm both when it is stimulated *through the nerve and when it is stimulated directly after curarization.* Very high

concentrations depress the contractions. The same worker (1949 *b*) has studied the pharmacological properties of antistin and benadryl (antihistamine drugs) and has observed some common properties in them.

Dutta (1948) showed substances like atropine, procaine, quinidine, pethidine and benadryl antagonised the action of acetyl-choline on body temperature of mice before and after adrenalectomy. He further discussed the role of acetyl-choline and adrenal glands on the control of body temperature

Burn and Dutta (1948) observed that these acetyl-choline antagonists also inhibited the constrictor action of adrenaline and sympathetic stimulation on the perfused vessels of the rabbit's ear They further showed that these substances, when perfused through the vessels, exerted an action like that of ergotoxine or priscol; they reversed the constrictor action of adrenaline. These substances themselves exert a constrictor action on vessels, and this constrictor action, like that of acetylcholine seen in the rabbit's ear vessels after 24 hours, was reversed under the influence of priscol The constrictor action of histamine in the rabbit's ear vessels was negated by the same substances which antagonised the action of adrenaline. The view that adrenaline dilatation and constriction were produced at the same site was discussed and the similarity in the action of adrenaline and acetylcholine was emphasized. The evidence was considered to support the theory of drug action by competition, and to suggest the mechanism of adrenaline reversal.

Roy and Gupta (1949) investigated the precise nature of the mechanism of the nervous control of the circulation in the systemic blood vessels of the frog by perfusing with varying concentrations of adrenalin hydrochloride and of acetylcholine, as being authentic representatives respectively of the adrenergic and cholinergic group of autonomic drugs. The observed facts seemed to suggest that (*i*) an autonomic drug was adrenergic or cholinergic in accordance with its concentration, for the time being, in the circulation being high or low, and (*ii*) all autonomic nerves were essentially of the same physiological nature, the character of their response as adrenergic or cholinergic being determined by the stimulus arising from high or low concentration of the causative agent in the circulation at the particular time.

The effect of tracheal stenosis on the lung of the rat was studied by Roy Cohn (1942). Obstruction of the broncheal tree of slight degree was not significant. When obstruction became significant, compensation took place by a slowing and deepening of the respiratory rate as well as an increase in the mean respiratory volume. Following the increase in mean thoracic volume, because of the stretch on the lungs, an increase in the amount of pulmonary tissue took place.

David, Krishnaswamy and Srinivasan (1940) studied inhibition of adrenaline oxidation. The role of phenolases in the oxidation of adrenaline and the inhibition of their action by stabilizers, namely, HCN and ascorbic acid, were studied. Ephedrine also appeared to act as one such stabilizers although in a limited degree.

Some experimental evidence had been given to prove the inhibiting effect of ephedrine on the oxidation of the system adrenaline-amine oxidase.

An attempt was made by Mukerji and Ghosh (1940), (1939-40) to find a simple test for efficiency of liver depending on the fact that some substances are conjugated in the liver with glucuronic acid and the amount of conjugation depends on the integrity of this organ. Also the damaged organ excretes the substance in its native form in proportion to the amount of damage of that organ (Mukerji and Ghose 1939 *a*, *b*). The urinary elimination of conjugated glucuronic acid by rabbits and dogs kept under conditions were estimated before and after administration of a glucurogenic drug, e.g chloral hydrate The daily level of conjugated glucuronic acid excretion was found to vary in two animals but an 'effective' dose caused in each a distinct rise in the conjugated glucuronic acid excretion. Chronic liver damage in these animals was produced by intermittent administration of toxic doses of *carbon tetrachloride. Effective doses of chloral hydrate was administered* to these liver-damaged animals and the rate of excretion of conjugated glucuronic acid was observed. During early stage of liver injury a tendency towards increased excretion of glucuronic acid was observed, though sometimes the rate of excretion was irregular. During the late stage of liver damage there was definite decrease in conjugated glucuronic acid excretion The rate of excretion of conjugated glucuronic acid was not regular all through These authors further observed that chloral hydrate, 200 mg. per kilo to apparently healthy dogs resulted either in no urinary elimination of free chloral or in elimination of free chloral in negligible amount.

In dogs with acute and chronic liver damage administration of chloral hydrate in doses of 200 mg per kilogramme brought about a significant well marked increase in the level of excretion of free chloral (Mukerji and Ghosh 1939 *c*) This difference in the level of free chloral elimination, the workers suggested, might be utilised as a measure of liver function in human cases The test, they observed, is fairly sensitive.

The difference in the rate of chloral clearance in blood in normal and liver-damaged dogs was studied by Ghosh and Mukerji (1941). The concentration of free chloral in the blood was determined by a comparatively simple but a sensitive colour test in apparently normal healthy dogs after administration of chloral hydrate in doses of 70 mg/kg, 150 mg/kg and 200 mg/kg The effects of identical doses of chloral hydrate on blood concentration was studied in the same dog after varying degrees of liver damage produced by oral administration of hepatotoxic doses of carbon tetrachloride. In both recent and long-standing cases of liver injury a significant increase in the concentration of free chloral in the blood was demonstrated (Mukerji and Ghosh 1939 *d*). The authors suggested that difference in the level of free chloral concentration in the blood, following the oral administration of a definite dose of the drug, might be used as a measure of the detoxicating efficiency of the liver.

Mukerji and Smith (1943) in their investigation of cyanide detoxication in rabbit and dog observed that cyanic administered either by subcutaneous or intravenous route, was almost completely converted in a comparatively short time into thiocyanate in the rabbit. A very large proportion of the quantity of cyanide injected could be recovered in the urine within the first 24-48 hours after injection or even earlier in experimentally induced diuresis. In the dog under identical conditions of treatment, thiocyanate recovery in the urine was very poor indicating a lack of complete conversion of cyanide into thiocyanate as in the rabbit. Usually less than 25 per cent of the injected sodium cyanide could be recovered within a period of 7 days

The effect of lecithin on haemolysis has been studied by Roy and Chopra (1941). The haemolytic action of the salts of quinine and optochin, according to them, seems to depend upon their acidity as these salts are easily hydrolysed A purified sample of lecithin has no action on the haemolysis caused by any of these salts The acceleration observed by previous workers has evidently been due to the free fatty acids present in impure specimens of lecithin which they used Purified lecithin has no appreciable action upon vibrio (El Tor) haemolysin but it has a marked inhibitory action on streptococcal haemolysin

The effect of surface tension on haemolysis has been investigated by Roy (1943). With none of the haemolysins examined by him e g saponin, sodium glycocholate, sodium taurocholate, sodium oleate, cyclamin and cobra venom, has surface tension been found to play any effective part in the initiation of the process of haemolysis. The same author (1943) also has observed that the fragility of erythrocytes belonging to certain species of animals varies inversely as their average diameter.

Toxicological studies on the synthetic anti-coagulant Dicoumarol (3. 3'-methylene-bis-4-hydroxycoumarin) showed a high potential toxicity for the compound as shown by extensive haemorrhages in the system, after oral administration. Further investigation showed that such reactions were not associated with any increase in the red cell fragility Bose (1945) found the thromboplastic activity of brain tissue was reduced in animals which had been fed with dicoumarol. This marked variation in the thrombo-plastic content of the rabbits fed with dicoumarol naturally suggest that some sort of physiological mechanism is probably at play, which alters or reduces the content of thromboplastin from the brains

Bose (1941) working on haemorrhages in liver diseases has observed that an evaluation of prothrombin level permits to foresee haemorrhagic tendencies in liver diseases and in jaundice. Administration of Vitamin K can cure such troubles if irreparable damage has not already taken place in liver parenchyma.

Jhala (1949) has assessed the value of Hippuric Acid synthesis as a liver function test. This test has proved useful to detect and estimated hepatic deficiency. It assesses detoxication function of liver adequately.

Kothari and Bhende (1950) have tried crude liver extract, refined liver extract and folic acid in 18 cases of pernicious anaemia of pregnancy. Majority of them have responded well to all the drugs

Bose (1949 *d*) has observed that purification and fractionation of liver extracts bring about an increase of the pernicious anaemia factor and do away with some factors more necessary for the maturation of erythrocytes in tropical macrocytic anaemia.

Hynes, Ishaq and Verma (1946) working on the effect of different diet and of iron medication on nutritional anaemias of Indian Army recruits observed that all forms of anaemia improved under influence of army diet but severe forms of anaemia were not cured without iron medication

Patel (1946) appraised the effects of crude and refined liver extracts in nutritional macrocytic anaemia and noted that pure liver extract gave optimum response in this anaemia; even small doses proved effective

The activity of a number of drugs to produce leucocytosis, with special reference to their possible value in the treatment of agranulocytosis has been studied by Das Gupta (1939).

Das Gupta and Napier (1939) examined the effect of spleen extract on the thrombocytes in a case of microcytic hypochromic anaemia with a very high platelet count. Intramuscular injection of 5 cc. of spleen substance per week for 5 weeks reduced the platelets markedly and subsequent treatment with ferrous sulphate brought the blood picture almost up to the normal level

The efficacy of the Indian-made liver extracts in the treatment of tropical macrocytic anaemia had been investigated by Das Gupta (1943). The results obtained with T.C F. 'whole liver' extract were similar to those obtained with the best crude liver extracts of foreign manufacture Cipalon also gave good results, though not as good as those obtained with T C F whose liver extracts The purified extract (T C.F. plain) gave results which definitely of a lower order and certainly not as good as those of highly purified liver fraction of anahaemin type

The role of synthetic folic acid (L. Casei factor) in the treatment of nutritional macrocytic anaemia had been reported by Das Gupta and Chatterjea (1946). They treated 8 cases of nutritional macrocytic anaemia with megaloblastic reaction of the bone marrow with the synthetic folic acid with appreciable improvements in most of the cases and in properly selected cases this treatment did not appear to be inferior in any respect to what is generally seen after potent liver extract, oral or parenteral The improvement was generally preceded by reticulocytosis. They obtained better effects with small daily doses of 20 to 30 mg given continuously than with interrupted big doses of 100 mg Further improvement attended the use of liver extract subsequent to administration of folic acid. Absolute achlorhydria cases showed lesser response than hypochlorhydria cases. Cases having total protein

more than 4.0 gm per cent showed better improvement than those whose serum protein was less.

Folic acid had also been used successfully, though with certain limitations, in the treatment of macrocytic anaemia in pregnancy by Das Gupta, Chatterji and Mathen (1949) In order to get its haematenic effect it should be given in doses of 20 to 30 *mg* per day for 30 days, in cases of pregnancy. The cases of dimorphic anaemia did not react very well to folic acid. Liver extracts, given parenterally or orally as proteolysed liver extracts, had been reported to have definitely a place in the treatment of macrocytic anaemia of pregnancy. In some cases it was found to be quite as good as folic acid.

Alterations of electro-cardiographic features brought about by *digitalis* was studied by Rahman (1941). The initial and most constant change in the electrocardiographic feature was found to be a depression in the S — T interval, followed later by an infarct type of QRS complex The T wave did not become inverted. On the contrary there was a tendency for the T wave to become more erect and pointed.

A comparative analysis of potency of twelve imported samples of injectable digitalis preparations was made by Chowdhury, Bose and Mukerji (1946). The data showed *deterioration of commercial preparations of digitalis*, even when stored in the form of powder in tropical climates.

Samples of digoxin have been found by Chowdhury, Ghosh and Mukerji (1950) to keep well even after a period of storage of 1 to 4 years under ordinary storage condition in the laboratory

It has been shown experimentally by Sen Gupta and Bose (1949) that the compressed tablets of Ascorbic acid are stable for a fairly long time

100 samples of cod liver oil and its preparations of both foreign and indigenous labels were analysed for their purity and vitamin A content by Iyengar and Mukerji (1939) Of these 64 samples failed to satisfy B.P. requirements and 25 samples were deficient in vitamin A and 4 were completely devoid of vitamin A.

A study of the keeping properties of *liquid preparations of ergot* in India by Bose and Mukerji (1941) revealed considerable deterioration of these preparations under the ordinary conditions of storage and retail distribution in this country. Bose, Dey and Mukerji (1942), therefore, attempted to find out means for checking the rapid rate of deterioration of liquid extract of ergot (B P 1932) in tropical climates and to stabilize it by addition of preservatives, anti-oxidants and reducing agents such as hydroquinone, metaphosphoric acid, merthiolate (sodium ethyl mercuric thiosalicylate) and ascorbic acid. Ascorbic acid was the only agent which had a demonstrable effect on the stability of liquid extract of ergot.

Dey, Bose and Mukerji (1942) observed that *ergometrine deteriorated in potency* to the extent of about 60 per cent in 9 months when exposed to ordinary conditions

in the light and heat of a pharmacy shelf in Calcutta Inside a refrigerator the rate of deterioration was very slow (8.29 per cent) but was none the less not insignificant.

Botanical, chemical and pharmacological examinations of Indian ergot were undertaken by Mukerji and Dey (1943) The analytical data clearly showed that Nilgiri Ergot satisfied all requirements laid down in the B P The total alkaloidal content of the imported ergot was found in six assays to vary between 0.010 to 0.110 per cent.

The stability of Thiamine hydrochloride was investigated by Gupta and Sen Gupta (1948). It was found to be fairly stable in aqueous solution at low pH, was not appreciably destroyed when heated for 45 minutes at 100°C and was unstable in presence of air Light did not accelerate the decomposition It was found that if its solutions with pH 4 were filled in colourless ampoules in an atmosphere of nitrogen or with as little air as possible in the ampoule and sterilized at 100°C. for 45 minutes there would be no deterioration Sen Gupta and Gupta (1949 *b*) reported the solubility of riboflavin (vitamin B_2) in water at different temperatures and the effect of various substances which might act as stabilisers for concentrated solutions of riboflavin. The same workers (1949 *c*) studied the problem of decomposition of ascorbic acid solution and observed that synthetic ascorbic acid solution underwent auto-oxidation. Heat and light did not increase the rate of auto-oxidation appreciably. A fairly stable solution could be prepared by taking precautions to exclude oxygen.

Das and Sen Gupta (1947) suggested some modification of the method of estimation of quinine sulphate to record its deterioration after long storage or by other means.

Sen Gupta (1948) evaluated a few economic poisons for use as insecticides

Serum phosphatase level in experimental liver and biliary damage has been worked out by Sehra, Chopra and Mukerji (1941) The phosphatase values in cases of biliary obstruction have been generally found to be greater than 25 Bodansky units. Rudra and Roy (1942) have observed that serum phosphatase in pulmonary tuberculosis is generally higher than that of normals, and that oral administration of large doses of vitamin C lowers the serum phosphatase of patients suffering from *pulmonary tuberculosis.*

Iyengar (1942 *b*) has investigated the relationship between thrombo-kinase and trypsin-kinase present in blood and has observed that thrombo-kinase and trypsin-kinase are two independent entities. Blood-kinase does not activate trypsinogen from the pancreas and differs in this respect from enterokinase. Enterokinase has been found to liberate active trypsin from the trypsin-inhibitor compound

The proteolytic activity of plasma from tuberculous and typhoid patients has been determined by Iyenger (1946 *a*) by studying the increase in non-protein-

nitrogen of the sterile plasma after incubation for 24 hours. In both the diseases a significant increase in the proteolytic activity over that of normal plasma has been observed. Iyenger, Sehra and Mukerji (1942 *b*) have reported that plasma trypsin is reduced considerably in cases of malignant growth. They have further shown that in diabetes and nephritis, there is either a tendency to increase or a marked increase in the content of free trypsin. In anaemia and thrombocytopoenic purpura both the free and total trypsin are practically absent.

The dextro-rotary hydrocupreidine derivatives were toxic in high dilutions and several workers appraised their comparative actions. Mukerji, Dutta and Ganguly (1942) studied the effects of some dextrorotatory hydrocupreidine derivatives on *paramaecium caudatum*. These were found to be powerful protoplasmic poisons and *in vitro* had lethal properties of a very high order compared to quinine. The toxicity of these derivatives on unicellular organisms increased with the increase of carbon atoms in the side chains. Iso-derivatives in this series appeared to be less toxic than their normal homologues though the difference in the intensity of action was not often very well marked. Laevorotatory hydrocupreines were more toxic to the ciliates than their corresponding dextro-compounds. The dextrorotatory hydrocupreidine derivatives were also found by Dutta, Chaudhury and Mukerji (1946) to be powerful spermicides and this action generally increased (up to hexyl derivative) with the addition of alkyl group in the side chain. No significant difference in behaviour was observed between these and their corresponding laevorotatory isomers.

De (1946 *a*) investigated the comparative pharmacological action of some alkyl hydrocupreidines The intensity of action was observed by him to increase in the homologous series of hydroquinidine and ethyl hydrocupreidine The sec-octyl hydrocupreidine behaved a little differently. All these derivatives in adequate doses had a depressing action on the plain muscle of the intestine and the cardiac musculature. The fall of blood pressure observed was partly due to the depression of the myocardium and partly due to the dilatation of vessels of the organs. A rise of pulmonary pressure was noted due to the constriction of pulmonary vessels. Pulmonary oedema was observed with higher members of the series.

The efficacy of a single dose of oil of chenopodium and tetrachlorethylene as anthelmintic for use in mass treatment has been tested by Hare and Datta (1939). Tetrachlorethyline has been found to be a superior drug. Tetrachlorethylene has also been shown by Maplestone and Mukerji (1940) to be a better drug than thymol for treatment of hookwork infection because of its lower toxicity, lower cost, greater ease of dispensing, less time taken in completing a treatment and greater efficiency.

The vital capacity of Bengalee students between 17 and 23 years of age has been found by De and De (1939) to be lower than that of the people in America and the British Isles.

Shaha (1939) has noted that the neuropoietin principle is normally present in the gastric juice and found it to be secreted by glands in pyloric end of stomach and by fundus.

The inhibition of acidity of gastric juice by spirit camphor was observed by Sinha (1948). With extractive 0 5 cc of spirit camphor could inhibit the free hydrochloric acid by 79.4 per cent and total acid by 82 7 per cent, in Pavlov I animal and for complete inhibition 2 0 cc were required The inhibitory response was greater when given without extractive.

Paul and Chatterjee (1944) observed that though the use of freshly prepared distilled or 'pyrogen-free' water reduced the pyrogenic reactions of the injections yet the use of acid or alkaline solutions, even if made with pyrogen free or fresh distilled water, was followed by some rise of temperature

The protective action of nucleic acid on the liver in carbon tetrachloride poisoning was investigated by Sundareson (1948). Nucleic acid given orally to rats 48 hours *prior to the injection of large doses of carbon tetrachloride had the effect of* reducing the liver damage as compared with that seen in control animals Infiltration of the centre of the lobule and portal canals with mononuclears was an outstanding feature in the livers of many of the experimental animals.

The question of control of sepsis in war wounds by pre-surgical treatment with bacteriostatic dressings and cod-liver oil sulphonamide emulsions has been discussed by Dikshit and Gardham (1945)

Prostigmin has been reported by Jacoby (1946) to have definite curative properties *in lathyrism, if given as injection for a sufficient length of time*

Krishnan, Mukerji and Dutta (1944) reported on the biological testing of transfusion material. Dutta and Chowdhury (1944) studied the effect of transfusion of serum in 'bled' cats.

Kaul, Mukerji and Chopra (1941) elaborated an indigenous mounting medium for microscopic work and investigated into the properties of some indigenous resins which might be used as a substitute for canada balsam.

Patel (1939) recorded the effects of various official and unofficial drugs on the blood pressure of dogs with special reference to cortical extract of suprarenal.

Myanesin (β-dihydroxy γ-(2 methylphenoxy) has been tried as an anticonvulsant in tetanus by Das and Roy (1949) in 17 cases. The relaxant action has been found to be definite and valuable but of a *very short duration The drug produces very* few complications.

Ghosh and Ghosh (1949) reported the result of treatment of 42 cases of scabies with Tetmosol, a synthetic drug of Imperial Chemical Industries.

Parpanit was tried by Sanyal (1949) in a case of Parkinson's disease with good effect on tremor and rigidity which was first noticed as late as the sixth day. There

had been no untoward effect. On stopping the drug, relapse occurred after a few days which yielded to further treatment with the drug. The effect of the drug was, however, transitory.

Harinasuta (1950) has treated 9 cases of amoebic liver abscess, two with pulmonary complications, with chloroquine with good response within a few days of medication. A progressive fall of blood pressure has been observed by the worker during its use.

Chaudhury (1949) has made interesting observations regarding specificity of choline esterase, the influence of temperature and irradication on its activity. The same author (1950) also has studied the effect of different chemicals on choline esterase and the reversible inactivation of choline esterase.

David and Krishnaswami (1942) while investigating the action of camphor, coramine and cardiazol observed that solucamphor depressed the heart muscle especially the auricle, due to its vagal effect and coramine produced depression of the rate and amplitude of the heart, whereas cardiazol or leptazol stimulated the heart to a smaller extent. On the respiration, all these drugs had a stimulant action on the centre.

Das and Chaudhury (1949) studied the action of Nikethamide *on circulation* and respiration observed that in small doses it produced little action on the heart of experimental animals In moderate to large doses a decrease in cardiac output was observed Nikethamide produced an initial fall of blood pressure *in almost* all animal experiments. The subsequent rise above normal level was practically absent. In human cases nokethamide produced neither a fall nor a rise of any significant degree It acted as a powerful respiratory stimulant and in large doses produced increased irritability resulting in convulsion.

Sen Gupta and Roy (1949) have studied the solubility of calcium gluconate in water at different temperatures, at which its supersaturated solution remains comparatively stable, and the effect of various stabilisers on the solution *etc.*

Individual variations in the absorption of drugs from the *gastro-intestinal tract* have been worked out by Chopra and Chopra (1945) Wide individual variation in response to drugs has been observed by them and they have collected extensive data in this connexion from biological standardization of drugs. The *absorption* of drugs is modified considerably in inflammatory condition of the gut, by rapid passage of meals through the intestines and nature of the drug

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VETERINARY SCIENCE

(1938-1950)

Edited by S Datta, F N I

I. Introduction

Veterinary Science in India has a long history prior to 1938, which is illustrated by the fact that the Indian Veterinary Research Institute celebrated its Diamond Jubilee in 1950 In 1938, Sir Frank Ware reviewed the progress of veterinary science during the preceding twenty-five years The review was published in the 'Progress of Science in India during the Past Twenty-Five years', which was brought out on the occasion of the Silver Jubilee of the Indian Science Congress Association in 1938

The present review covers in a general way the salient achievements and trends of research and development in Veterinary Science and Animal Husbandry during the period 1938-1950 It is by no means a compendium of the contributions of all the workers for this is not possible within the limits assigned to the sub-section There must, therefore, be omission, which the author hopes would be appreciated.

Previous to the period under review the work both in the Indian Veterinary Research Institute and the State Veterinary Departments had been concentrated on the problem of the conservation of animal health Subsequently, with effect from 11th March 1939 attention has been paid to matters of animal husbandry also During this period much importance was given to the study of animal feeding and breeding and the development of the poultry industry on scientific lines by the establishment of the Divisions of Animal Nutrition, Animal Genetics and Poultry Research at the Indian Veterinary Research Institute, Izatnagar. But the amalgamation of the Division of Pathology and Bacteriology into one has been a retrograde step which has adversely affected the work on Pathology. The Indian Council of Agricultural Research has continued to provide facilities both at the Central Research Institute and the states for work of fundamental, applied and developmental nature on various aspects of Veterinary Science and animal husbandry. The Council has also given a fillip to the study of various problems affecting the health and productivity of sheep, goats and poultry by providing Assistant Disease Investigation Officers (Sheep and Goat) and (Poultry).

During the same period steps have been taken to improve the standard of Veterinary Education in the country. A few more State Veterinary Colleges have been opened, bringing the total to nine and most of the colleges have been affiliated to Universities. To prescribe and ensure the maintenance of proper standard in veterinary education in the country, an All-India Veterinary Educa-

tion Committee has been established under the Indian Council of Agricultural Education A number of post-gradute courses have also been provided at the Indian Veterinary Research Institute, and facilities for imparting advanced training in special subjects have been increased at the Institute.

Steps have also been taken for the manufacture of improved biological products for efficient and expeditious control of bacterial and viral diseases, and for the application of the results of researches under practical field conditions under pilot extension projects

In the preparations of the detailed accounts of the achievements in the various fields of specialization which follow, much help, which is gratefully acknowledged, has been received from Dr K C Sen, Director, Indian Dairy Research Institute, and Mr. M. R Dhanda, Sardar Balwant Singh, Dr. N. D Kehar, Dr H. D. Srivastava, Mr. S. G. Iyer and Dr P Bhattacharya of this Institute Thanks are also due to Dr. J. M. Sen, Editor of Publications, National Institute of Science of India, for the consideration he has shown in extending the time for the receipt of the manuscript

II Pathology and Bacteriology

Viral Diseases

Rinderpest

It has been known that rinderpest the most serious scourge of cattle in India spreads through the ingestion of contaminated feeds and water, as well as by direct contact. It has now been observed that the disease can also be transmitted from the infected to the healthy by the expired air

The rinderpest virus was found to remain viable in the body of the tabanus fly for a period of 30 hours, but its role in the transmission of the disease does not appear to be of such significance

A cutaneous form of rinderpest has been reported from certain parts of the country, notably from Bengal where about 5 to 10 per cent of the affected animals showed small reddish eruptions on the skin which later dried up and formed scabs. The dried scabs are found to remain infective for fairly long periods It was estimated that the value of the hides from such animals became reduced by 40 per cent.

Attempts were made to dry rinderpest virus in leucocytes and spleen tissue emulsions by various methods. The prior treatment of tissue with cold acetone or acetone followed by ether appeared to be satisfactory but a considerable loss in the virus content resulted and drying *in vacuo* over calcium chloride appeared to be method of choice. Since dried Rinderpest vaccine would be easy to despatch and would retain its viability for a longer period, large scale manufacture using imported freeze-drying equipment is visualised.

A spray drying plant was devised on the lines of Hartley's method by which anti-rinderpest serum could be dried rapidly under reduced pressure at 40°C. without loss of potency. The stability of the dried product was found to depend on its residual moisture content. Preliminary observations on the precipitation of antibodies with alcohol were also carried out.

A severe outbreak of rinderpest in the cloven footed wild animals in a zoo was investigated. The animals affected were sambhar deer, *Neelgai*, hog deer and barking deer, and the mortality varied from 50 to 100 per cent The origin of the disease was attributable to air-borne infection from cattle from nearby localities.

(2) *Goat adapted rinderpest virus*

The viability of goat virus was studied under different environmental conditions. The cryochem dried goat tissue virus remained viable for a period of at least 3 months when stored at 0-5°C. and for 14 days when stored at 37°C. as against 7 days in the wet form.

Goat tissue vaccine virus usually used for vaccinating cattle in the fields had been suspected by some workers from time to time (Crawford 1947) to get disseminated to goats and thus establish in them an atypical form of the disease When goats and cattle were kept experimentally in close contact with animals vaccinated with goat virus no spread was observed

Goat tissue or goat blood virus has been used extensively for prophylactic vaccination of indigenous breeds of cattle, but the cattle of Madras evinced a severe reaction, loss of condition, reduction in milk yield and occasional death The simultaneous use of anti-rinderpest serum with the virus has thus become the routine in that state.

Similarly, goat virus alone has not been found to be very suitable for vaccination of buffaloes as these animals also show a severe reaction accompanied by reduction in the milk yield and abortion in pregnant animals. Thus in C.P. and Berar, Bachan Singh (1938) observed that whereas the indigenous breeds of buffaloes withstood inoculation with goat virus alone, there was reduction in milk-yield by 20 to 50 per cent in buffaloes of Murrah and Surti breeds, when similarly treated.

Rinderpest in sheep and goats, in this country is fairly widespread and outbreaks were reported from various states. Goat virus alone was used by some workers to protect sheep and goats and to check outbreaks but a small dose of antiserum seems indicated along with the virus to check severe reaction.

It has been observed that the administration of large quantity of anti-rinderpest serum with the goat virus results in a blocked-out reaction. The production of immunity however is not interfered with to any appreciable degree.

Under field conditions immunity in cattle vaccinated with goat-adapted virus lasts for about 3 to 5 years, while it has been proved experimentally that the

immunity was solid for a period of 12 years after inoculation with goat blood virus.

It was observed that cattle can be effectively immunised by scarification in the ear by means of a scarifier, the vaccine used being a paste of goat spleen tissue. It is doubtful however if the general practice of vaccinating by subcutaneous route will ever by supplanted.

Rinderpest Bull virus

It has been found that a strain of bull virus maintained at the Institute for the last 20 years or so, which has undergone about 230 serial passages in bulls has got attenuated to a considerable degree, while retaining its antigenic properties.

The strain when passaged serially in sheep for eighty generations showed definite attenuation for cattle as well as for sheep and goats, unlike the goat tissue vaccine which appears to have got fixed in virulence for goats and also to an appreciable extent for sheep, in both of which species it produces a severe reaction and is therefore not suitable for use in protective vaccination The sheep strain appears to be a better immunising agent for sheep and goats although further attenuation is now being attempted.

Rabbit-adapted virus

A virulent strain of rinderpest virus was passaged through rabbits in the hope of attenuating it sufficiently and evolving a suitable vaccine strain for use on highly susceptible breeds of cattle, buffaloes, sheep and goats, which react severely to goat tissue virus At the 55th passage in rabbits, the strain showed a marked degree of attenuation enabling a small scale field trial to be carried out on buffaloes in the Punjab with very encouraging results. With further passages in rabbits, the virulence of the strain was lost gradually till at 150th passage its infectivity was completely lost

Further attempts to evolve a rabbit strain in India were given up, as a result of the receipt of a lapinised virus (Nakamura's strain III) from China through the F.A O This strain appears to have reached attenuation to the desired extent and to have got fixed in virulence, as even after 795 passages in China and 180 further passages in rabbits in India, it still retains its avirulent character for cattle, buffaloes, sheep and goats and has proved to be a very suitable immunising agent for these species of animals. With the continued passage in rabbits however, there appears to be signs of its having slightly increased in virulence for these animals.

In order to see if this lapinised strain would undergo exaltation in its virulence as a result of serial passages in susceptible cattle if introduced in the field, the virus was serially passaged from bull to bull. No indication of any such increase in virulence or decrease in protecting power was observed during the course of 19 serial passages carried out in this connection.

The rabbit virus was also passaged from goat to goat to see if (i) it would result in an increase in virulence for these animals and (ii) to see the possibility of obtaining a cheap source of virus for the large scale production of the vaccine. During the course of 80 such passages the virus did not exhibit any change in its character.

Although it was found that inoculation of sheep with this virus renders them immune to rinderpest, the passage of the virus from sheep to sheep was not successful as the 5th passage virus did not prove infective to hill bulls

Rinderpest Eradication Plan

A scheme for the eradication of rinderpest from India at a reasonable cost, utilizing to the maximum the existing organization and budget grants in the States with minimum additional expenditure spread over 5-10 years has received the blessings of the Government of India and the Planning Commission This scheme visualises that what the different States in the Indian Union under the present conditions would jointly spend in 10-12 years on keeping rinderpest in partial check, if concentrated in a co-ordinated manner would result in 10 years in the total eradication of the disease from the country and effect a permanent saving thereafter of the huge loss in life and productivity estimated at Rs. 5 crores and the yearly expenditure hitherto incurred in its control It is proposed to use goat virus alone for the bulk of the stock and the lapinised virus for others which are highly susceptible to rinderpest.

As a preliminary to this mass inoculation some field trials with lapinised rinderpest virus alone were undertaken in some of the organized farms in the Punjab, U.P. and Madras to collect necessary data regarding the suitability of the product under varying conditions in different parts of the country.

Ranikhet disease

Ranikhet disease of fowls has been the greatest menace to the poultry industry ever since 1927 when the disease was first investigated in India. It was observed that fowls were easily infected when they were allowed to drink water contaminated with the virus Virus mixed with grains or virus in gelatine capsules when fed to fowls failed to induce infection. Susceptible birds contracted the disease when exposed for 3 hours or more to an ailing bird kept at a distance of two feet under conditions suitable for the play of air currents Haematological studies of fowls suffering from the disease revealed a marked leucopenia associated with a rise in neutrophils and fall in lymphocytes.

Evolution of the vaccine

Attempts were made to evolve a 'gel-absorbed' vaccine using aluminium hydroxide gel and suspension of spleen from the infected birds. Although many birds were successfully vaccinated by this method the results obtained were somewhat

irregular and because of its poor keeping qualities, the use of the gel vaccine was bound to be much restricted.

After Iyer and Dobson pointed out that a simple passage of the virulent virus in the developing chick embryos would result in attenuation of the virus, experiments using three field strains of the virulent Ranikhet disease virus, were carried out Out of the three, one strain showed signs of attenuation after 22 serial egg passages and a sufficient degree of attenuation was achieved at the 115th passage. With this attenuated strain of virus birds can now be safely protected against this deadly disease at the age of 6 to 8 weeks or over without their showing severe reactions. The issue of the vaccine was undertaken during the war to Military Poultry Farm and later to Civil farms from 1945 As the vaccine had poor keeping qualities and could not be transported over long distances except on ice, a batch of officers from the provinces was trained in the technique of its preparation in the same year and a few vaccine production centres were started. Today we have substations in Madras, Bombay, Bangalore, Hissar and Calcutta and the vaccine is also produced in many foreign countries such as Ceylon, China, Burma and Isreal. The vaccine strain has also been used in Portugal and Egypt, and has been distributed in recent years to many foreign countries It is remarkable that the vaccine strain has not shown any signs of further attenuation and its protective value after 220 passages is as good as it was after 115 passages when the strain was first used for field trials.

Birds immunized with this vaccine were tested to assess the duration of immunity after vaccination. It was observed that the immunity lasts for at least four years after vaccination, thus showing that the vaccine affords protection for the full utility period of a bird. It was observed that if vaccination was done in laying hens, egg production declined but returned to normal in 14-21 days. Vaccine stored as seed material at 4°C. remained viable for 3 years and 9 months the longest period so far tested. In a dilution of 1 10 made either with phosphate buffer solution pH 7.4, or amniotic fluid collected from uninoculated eggs on the 12th day of incubation, it remained viable for at least 212 days when stored in a refrigerator.

In order to improve the keeping quality of the vaccine, experiments were undertaken to dry the vaccine by the Cryochem process using an emulsion of the fluid and the chorioallantoic membranes of infected eggs. Vaccine dried in such a manner was found to remain viable without any appreciable loss intitre for 20 days at 37°C. but not for 46 days and at refrigerator temperature for 46 days but not for 120 days. Cryochem-dried vaccine stored in the refrigerator for one month remained viable for at least 15 days when sent to the field.

Foot and Mouth Disease

As compared to other countries foot and mouth disease in India is actually not so serious from the point of view of fatalities but it is enzootic and occurs

in all parts of the country and in all seasons. Thousands of animals that recover from the disease are rendered unproductive. It was not possible to devote any concentrated attention on this disease till 1943 when the Indian Council of Agricultural Research sanctioned a special scheme to be operated at the Indian Veterinary Research Institute.

Materials collected from the natural outbreaks in the field were typed against standard strains of O.A. & C. received from the Foot and Mouth Research Station, Pirbright, England. Examination of 169 field strains revealed that type 'O' was the most predominant. Attempts were concentrated on the evolution of a cheap and efficient vaccine. Alumina-gel-absorbed vaccine was observed to give variable protection from brew to brew on account of difference in the absorbing power of the 'gel'.

Several experimental brews of crystal violet vaccine were prepared on the lines of Graub *et al* and tested out on cattle, sheep and goats with satisfactory results, using a few hundred animals of the dairy herds of the Indian Veterinary Research Institute. The Immunity following vaccination has been tested up to 15 months Crystal violet vaccine prepared at this Institute was compared with the 'gel' vaccine obtained from Copenhagen and the tests indicated that the former is definitely superior to the foreign product. The only impediment to the application of the crystal violet vaccine on a mass scale is the prohibitive cost of production Experiments are contemplated to produce the virus in sufficient quantities by tissue culture methods.

(d) *Panting—a sequel to Foot and Mouth Disease*

A condition known as 'panting' which considerably incapacitates animals for work has been noted in a small percentage of cattle recovering from foot and mouth disease. It was observed that about 8 4 per cent of crossbred cattle and about 2 4 per cent of indigenous breeds of cattle in a number of military farms were panters. The condition is characterised by anaemia and is most severe in cattle in hot countries. Affected animals have a long rough coat. Panting is usually worse in the monsoon than in the hot dry season The rate of respiration is considerably increased in a panter even at rest, during heat on a monsoon day The outstanding pathological feature in panters is the reduction in the size of the thyroid gland with small vesicles and diminished storage of colloid. Albuminoid degeneration is noted in the muscles of the tongue and the heart. The animals develop a microcytic anaemia. It is suggested that the symptoms are due to the thyroid being too active for the animals' needs and the reduction in size being an attempt at adaptation. The panting condition may be due to (a) chronic anaemia (b) changes in the heart muscle and (c) an interference with the normal working of the pituitary-thyroid-adrenal mechanism which results in a more or less permanent failure of the heat regulating centre.

Pock diseases

It was observed that desiccated sheep pox vaccine remained potent for at least six months at refrigerator temperature as compared to the wet vaccine which lost its potency within one month. With a view to improve the current goat and sheep pox vaccine, experiments were undertaken to prepare a sensitised vaccine by treatment of the virus with antipox serum. The results are encouraging.

A new virus disease belonging to the pock group of viruses affecting sheep and goats was investigated. The disease whether in goats or sheep was characterised by high fever and appearance on the skin of hard nodules which subsequently were shed off leaving a 'punched out' scar. Pneumonia and mastitis were some of the complications of the disease The disease is of considerable economic importance as it causes heavy mortality amongst the infected animals and is difficult to distinguish from pox especially in the early stage. Experiments show that protection against this disease could be afforded by the application of the virus in the form of a 1·5 per cent suspension of nodules, to a highly scarified area in the inner aspect of the ear.

Vaccination of fowls against fowl pox carried out with pigeon pox virus has been observed to afford protection only for about 4 months and revaccination was indicated.

In order to evolve a more effective vaccine 3 parts of an attenuated fowl pox virus mixed with one part of pigeon pox virus and used as a vaccine. It was observed that although this mixture produced a satisfactory immunity it sometimes produced severe reaction in flocks with a lowered vitality. Attempts are now being made to evolve a live attenuated fowl pox virus by adapting it to the developing chick embryo.

Encephalomyelitis

In certain parts of the country army horses were observed to suffer from a nervous disease resembling the classical encephalomyelitis infection The etiology of the disease was examined and a virus isolated from some of these cases has been maintained by passage in horses and laboratory animals. This strain of virus was sent to the United States of America for specific identification against the three well-established strains existing in that country. It was observed that the strain was different from all the three existing strains and possibly it was a variant of Indian rabies virus. Further studies are in progress.

For many years in succession a disease simulating encephalomyelit and occurring in the cold season of the year was reported from the Government Livestock Farm, Hissar and also some sporadic cases in the farm at Izatnagar, Karnal and in Bihar. The main features of the disease were a non-febrile illness lasting for a few days, marked emaciation, gastro-enteritis and some stiffness in gait. A fairly constant post-mortem lesion was gastro-intestinal ulcer suggestive of rinder-

pest. The disease was transmissible by inoculation of brain material subdurally or instillation into eye or by insufflation into the nostrils but not by subcutaneous inoculation Inclusion bodies were detected within the Purkinje cells of the cerebellum. A vaccine prepared from the spinal cord of the affected animal was of value in controlling the disease.

A form of nervous disease amongst sheep and goats in Hissar was investigated. Transmission experiments failed to reproduce the disease in goats, sheep, rabbits or mice. A 4 per cent emulsion of the central nervous system in carbol saline gave satisfactory results.

Rabies

Attention has been paid during recent years to the development of a single-dose vaccine for protecting susceptible animals especially dogs against rabies, in place of the six-dose vaccine Dogra (1949) prepared vaccine using brain material collected from sheep infected with the fixed virus The final concentration of brain tissue in the vaccine was 25 per cent and carbolic acid 0 5 per cent It was incubated for 24 hours at 37°C and then stored at 24°C for 7 days.

Veeraghavan (1947) observed that rabies virus cultivated on a medium consisting of 1 5 per cent sheep brain material, 2 per cent sheep serum, 2 5 per cent glycerin and 0 15 per cent peptone gave a concentration of virus of 2,500,000 m l d's for guinea-pigs per cc

BACTERIAL DISEASE

Haemorrhagic Septicaemia

Virulent strains of the organism produced large, relatively opaque and fluorescent colonies as compared to the smaller and non-fluorescent colonies produced by avirulent strains of the organism Further it was observed that the organism of virulent strains were coccoid and capsulated

Naik (1949) observed that Pasteurella organisms occurred in the blood circulation 2¼, 13 and 36 hours after infection in rabbits, buffaloes and cattle respectively..Cultural and microscopical examination were not found to be as efficient as the biological test. Blood collected about 20 and 14 hours before death in buffaloes and cattle respectively was found to be invariably infective to rabbits. After death, the organisms continued to multiply in the bone marrow in rabbits during the first six hours and thereafter their number remained stationary up to 48 hours after which it declined. *Ctenocephalus felis* was found to transmit Pasteurella infection from rabbit to rabbit. Pasteurella organisms were found in the faeces of fleas fed on infected rabbits and then fasted for 48 hours; also for 72 hours in the bodies of infected fleas.

Nearly 4-7 per cent of healthy cattle were found normally to harbour *P. septica* in their upper respiratory tract. Due to lowered vitality as a result of exposure

to rains or due to other devitalising factors these carriers suffer from the disease and thus account for the spontaneous outbreaks of the disease.

Some experiments carried out to prepare a more potent H S Vaccine indicated that a vaccine prepared from a 24 hours old culture of virulent *P. septica* grown on agar slants and washed with phosphate buffer solution of pH 7.6 was better than a vaccine from avirulent strain grown in broth. Alcohol or formalin was a much better preservative than merthiolate, phenol, lysol or cresol. The immunity conferred was found to be solid for 7 months but not for 10 months. Attempts were made to evolve a strain of lower pathogenicity but with full immunising properties, by propagating a virulent strain of the organism in media containing penicillin, in embryonating eggs etc.

A study of the antigenic structure of *Past. septica* obtained from outbreak of the disease in the field showed that there were at least five different types.

Pasteurellosis in goats and fowls has been observed. In goats the organism was found to closely resemble Johne's group III and was highly infective to other species In healthy flocks of fowls nearly 4.06 per cent was observed to be carriers of *Past septica*

Penicillin was found to have synergistic action with sulphonamide drugs against this disease Penicillin was found effective in curing cases affected with Pasteurellosis.

An unknown substance present in liver kidney, muscle and blood serum was found essential for the growth of pasteuella organism in addition to a synthetic medium consisting of casein hydrolysate, tryptophane, cystine, glucose etc.

Anthrax

It has been confirmed that virulent strains of B' anthracis when cultivated in 60 per cent carbon-di-oxide atmosphere threw off rough avirulent variants which have good immunising properties. Vaccine prepared from the spores of such avirulent variants was safe for all species of animals and the immunity conferred was solid for 3 months and a high degree of resistance up to 10 months A vaccine containing 1 to 1½ million pores per cc, suspended in 50 per cent glycerine saline is now being issued from the Indian Veterinary Research Institute to the field. Experimentally it was shown that anthrax bacilli multiplied in heat sterilized soils at 25°-30°C. and with 20 per cent average moisture content. Within two weeks an increase of up to 30,000 times was observed and with cessation of growth the organism sporulated. Growth was also satisfactory in soil enriched with animal or plant manure as well as ammonium sulphate, lime and superphosphate. The addition of lime was found to be particularly advantageous. Presumably owing to the antagonistic action of soil bacteria, no multiplication of anthrax bacilli occurred in unsterilised soil or water.

Brucellosis

A survey of the incidence of the disease over a period of six years indicated that this disease occurred in all Indian farms and in many rural and urban areas. *Br. abortus* has been found to be the main organism causing the disease although some intermediaries between abortus and melitensis have been observed. Occurrence of *Br. melitensis* in sheep and goats has been recorded Diagnosis of the disease is carried out by a thick coloured antigen for a quick test and an ordinary antigen for tube test. Vaccination with coltin strain 19 of *Br. abortus* has given satisfactory results.

Black Quarter

Minett (1945) observed that at the time of death of an animal due to black quarter the organisms were usually in the vegetative stage and continued to multiply in the body some hours after death When the organism have multiplied some 50-230 times, they sporulate. Young sheep, showed a good deal of resistance to the disease. Although large numbers of spores given intravenously produced no infection, subcutaneous inoculation produced no infection, subcutaneous inoculation produced the disease better than spores given intramuscularly. By means of calcium chloride, latency has been determined up to 25-40 days in subcutaneous tissue and up to 20-25 days in muscle tissue. By cultural methods spore latency was determined up to 25 days in bone marrow and 18 days in lymph glands It was observed that the higher glycogen level of well-fed sheep is concerned not with spore germination but with multiplication of the bacilli after spores have germinated. It was also observed that a nutrient broth containing 0 05-0 1 per cent cysteine hydrochloride was suitable for primary isolation of the organism Media containing 0.5-1 per cent glucose broth containing 0 05 per cent cysteine hydrochloride or media containing extract of liver digested with concentrated hydrochloric acid was found suitable for large scale growth of *Cl. chauvoei*

Tuberculosis

A rough idea of the incidence of tuberculosis in cattle in India was studied by periodic examination of carcasses in the slaughter house and by large scale tuberculin tests in certain parts of the country The results showed that the incidence was greater in Northern India notably the Punjab The method of preparation of tuberculin was modified by the introduction of synthetic media by which a more reliable and standard product was obtained.

A number of tuberculous specimens originating from human beings were examined. Forty-two strains were isolated and all conformed to the human type. A number of samples of milk and cream collected from cows positive to tuberculin test at Hissar and Dehra Dun and a few samples of cream from four milk distributing centres in Bombay and Madras were subjected to biological test for the presence of tubercle bacilli but none proved positive. Investigation of tuber-

culosis in goats revealed that goats are highly susceptible to bovine type of the organism and that the lesions are confined mainly to the lungs and the associated glands. The single intradermal test in the caudal fold was found superior to the double intradermal test in the neck. Examination of 451 carcasses of pigs slaughtered in Bombay revealed lesions of tuberculosis in 17 per cent of them The organisms isolated from these cases showed that the majority had infection of the human type and a few were infected with the bovine and one with the avian type The existence of tuberculosis in poultry has been established and the organisms isolated conformed to the standard Avian strains of tuberculosis

Vaccination of calves against tuberculosis was attempted using 'Vole' strain of tubercule bacilli, with encouraging results. Experimental vaccination of rhesus monkeys with this organism however did not indicate the development of an appreciable degree of resistance against tuberculosis.

Johne's disease

A survey of the incidence of the disease carried out in the various farms showed it to be prevalent in almost all the herds in Madras, Mysore and Orissa. Johnin prepared from organisms grown on synthetic media by substituting ammonium malate for asparagin as a source of nitrogen, gave very satisfactory results An outbreak of Johne's disease amongst goats at Mukteswar was investigated.

Mastitis

Preliminary investigation on the aetiology of mastitis has shown that *Streptococcus agalactiae* was the most common cause of chronic mastitis of contagious type and *Staphylococcus aureus* of acute or subacute mastitis. Considering the importance of this disease from the point of view of dairy industry a scheme for carrying out intensive studies on this disease has been prepared and submitted to the Indian Council of Agricultural Research.

An epizootic form of mastitis in goats was investigated The infection was observed to run a peracute course for several days and then abated, assuming a somewhat chronic form. Bacteriological examination showed the presence of *Corynebacterium pyogenes* as well as a form of virulent strain of Pasteurella This disease is of great economic importance and further investigations are in progress. A specific mastitis of goats associated with *Corynebacterium avis* has been frequently reported and with *Staphylococcus aureus* as well

Contagious Bovine Pleuro-pneumonia

This highly fatal disease of cattle was not known to occur in India until the last war when cases were first seen in Assam Transmission of the disease was easily achieved by subcutaneous inoculation of lung exudate from natural cases. The exudate was found non-infective to rabbits, guineapigs and goats. The virus was found filterable through Berkefeld, V.N , Seitz E.K. and Chamberland and L3

filters and was cultivable in Benett's serum broth, pH 7 4 from filtrates of local lesion exudate Cross immunity tests carried out with strains obtained from Australia and South Africa where the disease has been occurring since years, showed that all the strains were identical, culturally and serologically.

The use of pleural exudate from affected cases stored at 5°C. for 3 months and inoculated at the tip of the tail gave satisfactory protection Such a vaccine used in the field in Assam was found to be safe and enabled to control of the disease.

Contagious Caprine Pleuro Pneumonia

During recent years many outbreaks of pleuro-pneumonia in goats causing heavy mortality were investigated Sheep and cattle were not affected Material collected from three different parts of the country proved on cross immunity test to be immunologically identical The causative organism has been isolated and cultivated artificially. Cultures of the organism from fresh cases were found to be very virulent. Successive cultivation on artificial media resulted in attenuation to such an extent that after 40 serial passages they were useful as a vaccinating agent Experimentally it was observed that the 70th generation of the organism in artificial medium when inoculated subcutaneously at the eartip conferred dependable immunity for at least six months.

Pneumonia in goats

A variety of agents have been found to cause pneumonia in goats Besides pleuro-pneumonia described above, pneumonia in goats is caused by the coryne-bacterial group of organisms, parasitic pneumonia by *Varestrangylus pneumonicus* and a fourth type of pneumonia is apparently associated with Pastourella infection. Further investigations on the fourth type of pneumonia are in progress

Anaerobic infections of sheep and goats

A condition in sheep resembling braxy was investigated in certain places in Madras and Bihar. *Cl. septicum* was isolated from cases in Madras and *Cl chauvoei* in Bihar.

What appears to be true enteretoxaemia due to *Cl. welchii* type D was proved to occur in the grazing tracts in Baroda States The disease was seen after the first rains when animals are allowed to graze freely. Filtrates of the intestinal contents from the affected sheep were found to be toxic to experimental animals

Strangles

Investigations on this disease as it occurred in two large remount depots and certain horse breeding farms in the Punjab showed the annual incidence of the disease to be about 50-90 per cent in young stock with a mortality of about 50 per cent of the animals affected Mules were found to suffer more severely. The disease was easily produced experimentally in foals by instillation of 24 hours

broth culture of the streptococcus isolated from affected cases The organisms isolated from these cases conformed culturally and biochemically to Edwards type B and Barele's Type 4.

Equine abortion

Experiments were carried out to improve the current vaccine against *Sal. abortus equi* infection of horses. It was found that addition of 2 per cent potash alum precipitated undesirable toxins in the broth culture. After precipitation of the toxins the filtrate treated with formalin was sufficiently detoxicated to freely used in ponies without any ill effects.

Corynebacterial Infections

C. equi infection in foals causing pneumonia was investigated. No toxin could be demonstrated in culture filtrates of the organism grown in different media and under conditions conducive to toxin production.

The occurrence of *C. ovis* infection in seven goats all at one time indicated some common source of infection Investigations on the toxinogenic properties of the organism with the ultimate object of evolving a suitable vaccine or a toxoid are being taken up.

Paratyphoid infections

A number of salmonella were isolated from pigs during the course of an outbreak of anteritis in a large piggery in Northern India. Investigations showed that they belonged to four different types viz., *Sal. Bovismorbificans, Sal. derby* and *Sal. anatum.*

Several strains of salmonella isolated from chicks and adult birds were typed. They were found to belong to group D of Kuffman-White scheme. Most members of the salmonella group being pathogenic to man, to isolation of these organisms from pigs and poultry in this country is important from the public health point of view.

Epizootic Lymphangitis

The only symptom noticed in what apparently appeared to be outbreaks of epizootic lymphangitis among army horses at Meerut, Saharanpur and Lucknow was conjunctivities with lachrymation or a muco-purulent discharge at the inner canthus. In some the parotid and submaxillary glands showed enlargement and suppuration. *Cryptococcus farciminosus* was isolated from the suppurate material. As the disease progressed the bronchioles and lungs were affected Measures for the control of the disease were laid down and destruction of all affected animals advocated as required by law.

An outbreak of the disease among the cast army horses obtained by the Haffkine Institute, Bombay was also investigated and control measures adopted.

Circling disease (Listerellosis)

A condition known as circling disease amongst sheep occurring soon after the first showers after a period of draught and when the grass is sprouting was investigated. The disease lasted for about a month and was sporadic.

The disease was characterised by inco-ordination, trembling, erratic movement of the head and a characteristic circling movement of the head followed by coma and death. Post-mortem examination revealed acute congestion of hippocampus, spleen, liver and kidneys. Gram-positive organisms were observed in the brain tissue culture. The causative organism was found to resemble listerells in respect of morphology, cultural characters and serological reactions.

Canine fever (Rickettsiosis)

The presence of Vickettsial bodies in the monocytes in the blood-forming tissues of affected dogs has been amply demonstrated The disease was easily transmitted from the affected to the healthy by blood inoculation Sulfapyridine combined with tryparsemide was found to be of great value

Melioodosis in man

Bacteriological examination of blood and urine samples from a sepoy who was suffering from pyrexia of an undulent type from about 6 months and suspected to be infected with *Br melitensis* led to the isolation of *Pfeifferella whitmori.* The disease occurs in the Far East and is usually carried through the agency of rodents. The patient had a history of field service in Burma and the Far Eastern colonies. The possibilities of this disease being introduced into India by the movement of troops should be looked into.

General Diseases

Horn Cancer

Cancer of the horn which is commonly met with in Indian cattle was studied. Materials from fully developed cancer cases showed on histopathological examination, squamous celled carcinoma with typical 'pearls' or 'cell nests'. Signs of metastasis in lungs have also been noticed showing the malignant character of the growth. Search for *Rhinosporidia* gave negative results.

Millet poisoning in man and animals

A sample of millet fat 'Varagu' which was found responsible for millet poisoning in Madras in people consuming this cereal in place of rice, was studied for its toxicity. Experiments showed that the fat had no effect on cows when administered orally or by injection but toxic symptoms were observed in dogs. These symptoms disappeared usually after 24-36 hours. The lesions were confined to the intestines which showed diphtheritic deposits. Histopathological examination revealed neuronophagia and nuclear degeneration in the nerve cells in the brain.

Equine embryonal nephroma

An interesting record has been made of a rare condition in horse. A 9-year old Australian mare suffered often from colic and haematuria, later developed icterus and died. On post-mortem there was a larger number of neoplams adherent to and apparently growing from the peritonium and the abdominal viscera. The growth varied in size from that of a pea to a large-sized melon. The largest tumor weighed 62 lbs. In all about 1000 new growths were present in the abdominal cavity The left kidney was probably degenerated within the main tumor mass. The tumor on miscroscopical examination could be divided into the components (a) thick strands of connective tissue and (b) areas consisting of highly cellular tissue, considering the clinical and autopsy records and the complex nature of the growths and the presence of sarcomatous cellular moiety and demonstrable states in the derivation therefrom of more or less drawn out glandular structure the tumor had been diagnosed to be one of the embryonal nephroma.

Angiosarcoma in a mule

The finding of this rare tumor in the thorax of a mule was reported. The tumor was widely disseminated in the thorax and the parenchyma of both the lungs and were pea- to grape-sized Invasion of the heart and aorta was noticed.

The tumor consisted of polyhedral cells which were enlongated sometimes with a clear cytoplasm and vesicular nucleus, with two distinct nucleoli Capillary formation was evident in some portions. Fatty degeneration and fragmentation of muscle cells were present.

III. Biological Products

The enormous livestock population in India and tremendous loss of lives due to diseases have always placed before the livestock immunologist the urgent need to evolve prophylactics for the prevention of such losses. There is therefore no greater achievement in the sphere of livestock health services than the advances made in this direction by the research workers of the Indian Veterinary Research Institute and the Biological Products Division of this Institute have been responsible for the manufacture of sera and vaccines evolved by them and supplying a large number of doses to field workers during the last twelve years.

1. *Products supplied*

This Division has undergone the greatest evolution during the last ten years. Before 1940 this Section was the Imperial Veterinary Serum Institute and entrusted with the manufacture of biological products e g Anti-rinderpest serum, Anti-haemorrhagic septicaemia serum, Anti-anthrax serum and Blackquarter serum. In 1940, manufacture of Haemorrhagic septicaemia vaccine, Fowl-pox vaccine, fowl-cholera serum, fowl-cholera vaccine and Blackquarter vaccine was

started. Later on, Fowl-pox and Anthrax-spore vaccines were also added. It is hoped that some more products will be manufactured here after the Section is modernised and some of the modern biologicals against rinderpest and rabies will also be produced.

During the early part of this period, the work in this line was centred at Mukteswar and Izatnagar only. Researches on rinderpest was given the top priority and engaged the attention of workers Two agents viz. goat-adapted rinderpest virus vaccine and anti-rinderpest serum were used intensively in field campaigns to combat the major scourge of cattle in India Later attention was focussed on other bacterial and viral vaccines, which have since then undergone a series of notifications Improvements in the processing of blood have resulted in appreciable increase in the yield of serum Establishment of cold rooms ranging from 32°F to 60°F has facilitated the storage of sera and vaccines The increased intensity of production during the war and partition years has led to many interesting observations regarding the technique of immunisation of buffaloes for anti-rinderpest serum manufacture and a clear picture has emerged of the wide variation existing in their susceptibility to the virulent bull-virus and various dose rates potent serum inoculations. It has been shown that effective vaccination against rinderpest does not consist in the inoculation of goat-adapted virus alone as such a vaccine may elicit unfavourable reactions in buffaloes and certain other susceptible breeds of cattle e g Ongole, Amritmahal etc. and therefore goat blood virus vaccine with serum still enjoys good reputation as a preventive against rinderpest preferable to goat-adapted-virus vaccine alone Goat-adapted virus with a small dose of serum enjoys reputation as a good prophylactic for buffaloes, sheep and goats Passive immunisation with rinderpest serum alone method is still in vogue in India The immunity conferred by this method lasts for about 10 days for hill cattle and *indigenous breeds of high susceptibility, the dose being 10 cc per 100 lbs body* weight In case of other cattle and buffaloes, the dose rate cannot be fixed owing to their varying susceptibility. On what this susceptibility depends it not known but some very valuable information is available in this respect from the studies on blood grouping of cattle in India (Balwant Singh) which shows clearly the increased susceptibility of some particular breeds of animals of specific blood groups.

In rinderpest immunization, especially by serum-simultaneous methods, care *should be taken to take account of the potency of serum Two different types of* anti-rinderpest serum are manufactured at this Section viz anti-rinderpest serum, special (Whole potency is more than 80 per cent on biological tests) and anti-rinderpest serum, ordinary (less than 80 per cent). Serum either special or ordinary, *loses its potency in storage and under plain conditions* (where the temperature ranges from 60-110°F) it becomes useless after one year. However if it is stored at freezing temperature, the potency, though lowered a bit, remains quite high even after 2 years. The special serum is generally suitable for imported foreign as well as cross-bred cattle.

The immunity conferred by the serum simultaneous method of immunization is life-long. Care, however, has to be taken not to overdose with serum which usually results in 'blocked out' reaction and breakdown in the immunity is frequent in such cases

During these years of serum manufacture, it has been notified that immunisation against rinderpest is best done during the cooler part of the year which aids in the production of a serum of higher anti-body titre than during hot and humid months. Buffaloes which are the serum donors have a weak thermo-regulatory system and with the increased difficulty in the heat dissipation, antibody formation in hot humid days is inhibited.

The most important drawback with a live spleen tissue vaccine is its short life It cannot be used for more than 4-6 days after its manufacture, and when emulsified it cannot be used even 12 hours after manufacture Efforts have been made by some workers to manufacture a dry pill-vaccine with encouraging results. It is envisaged that freeze-drying will solve these practical difficulties.

In the field of manufacture of other types of sera improvements have been few and the manufacture is based on the knowledge available from researches carried out by workers at this Institute and abroad

Anti-anthrax serum for animals is produced from buffaloes with an avirulent organism It has a protective value and confes-immunity for a period of only 10 days. The dose is 15 cc. for all adult cattle.

Blackquarter serum is both anti-toxic and anti-bacterial and is effective against *Clostridium chauvoei* as well as *Clostridium septicum*. The immunity conferred lasts for about 10 days and 15 cc is the dose.

Haemorrhagic septicaemia serum is also a prophylactic agent with a protective duration of 10 days The dose is the same as that of Blackquarter or anti-anthrax serum

Fowl Cholera serum is also manufactured but is not so much in vogue these days.

Except anti-rinderpest serum, which is also manufactured on a small scale at the Serum Institute, Ranipet, Madras, none of these sera are manufactured in India outside the Indian Veterinary Research Institute.

Vaccines

Haemorrhagic septicaemia vaccine is manufactured here and recently this is also being manufactured at state serum Institutes and vaccine laboratories at Lucknow, Bombay, Madras and Mysore. Formerly heated broth cultures (of *Pasteurella septica*) were being used and latterly lysol-killed whole culture This vaccine though effective against haemorrhagic septicaemia in cattle has a poor keeping quality and the duration of immunity conferred by it is very short. Hence in spite

of the demand, it is far from being perfect and efforts are being made to evolve a better and more potent vaccine. A better vaccine has been manufactured also at times e g agar-wash vaccine, consisting of a thicker suspension of organisms grown on agar slope. This vaccine is reported to be a better one Similar efforts have been made to manufacture a vaccine against fowl cholera as well. The dose is 5 cc.

Blackquarter vaccine is a poly-valent vaccine, being a formolised whole culture of *Clostridium chauvoei* and *Clostridium septicum*. The toxid of these clostridia and their cellular antigen afford solid immunity for about a year when injected in 5 cc. dose and is reinforced by a second dose The vaccine keeps for 4-6 months.

Anthrax-spore vaccine is a new addition to the list of recent vaccines Evolved on the lines of Sterne this vaccine consists of a live suspension of avirulent bovine anthrax spore in 50 per cent glycerine. The vaccine is used in 1 cc. dose (consisting of 1 million spores/cc) and has been found to be a very good vaccine against anthrax in elephants, cattle, buffaloes, sheep and goats

Bovin abortion vaccine consists of a 1 per cent live bacterial suspension of Cotton strain of *Brucella abortus*. Injected in 5 cc dose, it affords immunity against contagious abortion This vaccine is extensively used for immunising calves

Sheep pox, Fowl pox, mixed strepto-coccal and equine-abortion vaccines are also manufactured but much more work is necessary to perfect them

Apart from these sera and vaccines, there are some important diagnostic agents which are manufactured here, e g, Mallein for subcutaneous and intradermal-Palpebral tests — Tuberculin subcutaneous and concentrated, Johnin and Avian tuberculin concentrated Some efforts are being made for the manufacture of purified protein derivative from tuberculo-proteins in broth culture both by dialysis and high speed centrifugation.

Diagnostic agents like Brucella abortus antigen, Brucella abortus quick antigen and Salmonella abortus antigen are also manufactured These products have been perfected very recently

2. *Service during World War II*

Biological Products Division, *Indian Veterinary Research Institute has been* supplying biological products according to the demands of the country. Thus it was able to meet the heavy demand during World War II

During the war period (1939 to 1945) 2.2 crores doses of biologicals were issued. The average annual rate was 41 5 lakhs doses as against 13 1 lakhs doses in previous years.

3. *Field of supply*

The supply of biologicals was not limited to the Defence and Civil Services in

India but also to various neighbouring countries e.g., Afghanistan, Iran, Iraq, Egypt, Ceylon, Burma, Malaya, Siam, China, Tibet, Nepal, etc.

4. Partition of India

The responsibilities of the Biological Products Section were further increased at the time of the partition of India with the phenomenal exodus of people from partitioned areas with their livestock. As a result of these indiscriminate movements severe epizootics raged in the areas in and around Punjab (India), PEPSU, Bikaner, Himachal Pradesh and Uttar Pradesh The emergency necessitated a quick and prompt supply of various biologicals to these areas and the burden was fully borne by this Division.

In spite of the partition of India, and stoppage of supply of biologicals to Pakistan, the production of biologicals has considerably increased. The price of live stock has increased tremendously. The acute shortage of food and importance of bullock power and need for more production have infused a spirit of animal-mindedness and this awakening has led to the increasing use of veterinary biologicals for the protection of animals against diseases.

IV(*a*) ANIMAL NUTRITION

The history of research in Animal Nutrition in India dates back to 1921, when Dr Warth initiated work on the chemical composition and nutritive value of feeds at Pusa. A real stimulus to the detailed study of all aspects of animal nutrition in India was given by the establishment of a Central Animal Nutrition Laboratory at the Indian Veterinary Research Institute, Izatnagar. The laboratory was opened in 1939 and during the last 12 years, it has carried out, besides studies on chemical composition and nutritive values of feeds, investigations on fundamental problems like requirements of proteins, minerals and vitamins of Indian livestock as well as on the problem of malnutrition and various diseased conditions. Valuable work on certain aspects of animal nutrition was also carried out at Lyallpur and later at Ludhiana in the Punjab. In addition, work on problems of regional interest was carried out under the auspices of Indian Council of Agricultural Research at Coimbatore, Dacca, Sabour (Bihar) and Bharari (U.P.). Work at the Central Institute as well as the other stations has brought out clearly that the problems of animal nutrition in such a large country as India varies a good deal from region to region and as such a single laboratory for the whole country is not adequate to cope with the diverse problems. Accordingly the opening of four regional stations for animal nutrition survey work was advocated and agreed to in principle by the Government as early as 1945, but financial stringency has prevented the opening of these stations. It is hoped that such stations will be established in the near future.

The figures for the availability of feeding stuffs have revealed that the shortage

of food for animal consumption is very great in the country (Das Gupta 1947; Sen and Ray 1941, Sikka 1947, Swarup 1947; Wright 1937). More comprehensive calculations reveal that the shortages of protein and energy are of the order of 60 and 40 per cent respectively (Kehar 1946; Williamson *et al.* 1947) It may, therefore, be said that malnutrition of livestock in India is the greatest single factor in the degeneration of livestock in India and unless sufficient feeds of the proper quality are made available, no improvement of the stock is possible The improvement in milk yield (Kartha 1942) or in growth rate of calves (Rajendra 1949; Ray Sarkar 1948) as seen when balanced rations are fed to village cattle, gives practical demonstration of this conclusion.

Subsidiary Foods

Since its inception the Animal Nutrition Division, Indian Veterinary Research Institute has been aware of the huge gap between the available and required amounts of feeding stuffs and considerable work has been carried out by Kehar and associates to find new sources of fodder and concentrate. During the period under review a number of products which are at present being wasted, have been shown to contain sufficient nutrients which after simple processing can be utilized as cattle food. Thus mango seed kernel, jaman seed, panwar seeds, tamarind seed, entrails of dead animals, fish meals and blood meal have considerable protein content and can be used to replace grains in concentrate mixtures Similarly after processing, the coarse grasses like kans, munj and the plants Kantiara, *Typha latifolia Edgent*, sugar cane tops and panewar straw can serve as roughage Bye-products like groundnut husk can also be fed to a certain extent during famine conditions. Baggo-molasses (1 part of bagassee screening and 2 parts of molasses) has been shown to be able to replace gram husk in village cattle rations Further unpublished work in the Animal Nutrition Division has shown that Mahua cake and mahua flower, sun hemp seed, rain tree peds, bajra and coffee husks and tree shed leaves can be used as animal foods (Kehar and Chanda 1944, 1945, 1947 *a*; Kehar Sahai and Chaudhury 1948, 1949, Kehar and Murthy 1950, Patel and Shah 1944, Kehar and Sahai 1949; Negi 1949, 1950, Millen 1946, Kehar 1944 *a*, *b*, 1948, Kehar and Negi 1950, Ray and Talapatra 1945).

Chemical Composition of Food Stuffs

Accurate information on the chemical composition of livestock foods is an essential prerequisite for determining their nutritive value. Data published in India have been compiled in 2 reports (Lander 1942 and Sen 1946). Later work include the examination of grasses and other foods in different parts of country (Athawala 1944; Brito-Mitnnayagam 1950; Chandrasekhar *et al.* 1947, Daji 1943; Kehar, Ray and Agarwal 1947; Menon 1939; Millen 1946; Rao 1944; Talapatra and Goswami 1949). A good deal of stress has been placed by several laboratories

on the chemical composition of crops and grasses as affected by maturity and number of cuttings (Chatterji and Ali 1947, Chet Ram and Ray 1943; Dharmani and Singh 1946, Kehar and Talapatra 1947, Khan and Bhatnagar 1945, Saimi 1940). Studies reveal the best times for cutting fodder for conservation purposes. A few papers have dealt with the effect of manures and crop rotation on the composition of plants (Parr and Bose 1944, Rao, Desai and Tejwani 1949) Though chemical composition of a feed gives a rough idea of its feeding value, feeding trials with experimental animals are required for ascertaining the availability of the nutrients present A large number of these trials have been completed and results up to 1946 have been tabulated in the Indian Council of Agricultural Research Miscellaneous Bulletin No 16 (1942) and Indian Council of Agricultural Research Miscellaneous Bulletin No 25 (1946) Later work deals with Assam grasses (Talapatra and Goswami, 1949) berseem at different stages of maturity (Das Gupta 1942, 1943, 1945, 1947 *a*, Chatterji and Talapatra 1940) rice straw grains and grain bye-products, giant star grass, tree leaves and sunflower plant (Lander and Dharmani 1944, Pandisekere *et al* 1948, Mathur and Roy 1943, Ray, Zubairy, Kehar, 1947, Sen 1942; Chet Ram and Ray 1943, Momin *et al* 1943, 1947)

Conservation and Processing of Feeds

Conservation of feeds is not practised by the average cultivator in India In view of the shortage of feeds especially during the summer months, it is necessary that all possible steps for the conservation of surplus monsoon grasses and fodders should be practised The majority of papers in this section deal with silage making. Sethi (1940) discusses the problems of ensilage in heavy rainfall areas, and silo pits suitable for a farmer have been described (Gupta 1948, Vaugh 1945) Sugar cane topes when properly ensiled yield quite good material (Kehar and Zubairy 1951, Ray and Chet Ram 1945) Paddy straw or shed tree leaves when ensiled together with lucerne or berseem yield a very palatable product having good nutritive value

Rice straw has always been regarded as a poor feeding stuff. This has been shown to be mainly due to its high content of potassium and exalate (Chatterji 1945; Sen, Ray and Ray Sarkar 1944, Talapatra *et al* 1942, 1949). Through water washing a large amount of the obnoxious salts can be removed so that the resultant product is made more nutritious (Ray 1942 *a*, Ray *et al*, 1947) A better method in improving the nutritive value of this as well as of other coarse fodders is treatment with dilute alkali (Kehar and Ray 1947, Sen, Ray and Talapatra 1942; Zubairy and Ayyar 1949).

Energy Metabolism

The requirement of energy by different species of livestock under different physiological conditions has got to be studied before a scientific balanced feed can

be formulated. So far the energy requirement of Indian livestock has been and is still being calculated on the basis of formulae established for European and American breeds of livestock A beginning has been made by the introduction of indirect calorimetry (Mullick 1945) to estimate the energy requirements of Indian cattle. Such studies reveal that Indian cattle may require 25 per cent less calories than that advocated by foreign workers The use of fuel calorimeter for determining the gross digestible energy of feeds has also been suggested (Dharmani, Mangat and Lahara 1946).

Protein Metabolism

It has been stressed before that the shortage of protein supply to Indian livestock may be as high as 60 per cent or even higher. This calculation has been based on protein requirement figures given by foreign workers In order to check whether such foreign figures hold good for Indian cattle as well, a systematic study was undertaken to determine the minimum protein requirement for maintenance. This involved the formulation of a 'nitrogen-free-ration' on which cattle can be kept for suitably long periods for observation The endogenous urinary nitrogen was shown to be 0 020 g/kg body weight i e 50 per cent lower than the lowest recorded figure for cattle (Kehar, Mukerj and Sen 1943) Metabolic faecal nitrogen was shown to be proportional to the dry matter content of faeces. From these investigations it was apparent that the protein requirement of Indian cattle for maintenance may be substantially lower than that already in vogue. Experiments towards this end with practical feeds has shown this to be true, the maintenance requirement being 0 4 lb of digestible crude protein per 1000 lb body weight — a value 30 per cent lower than that by foreign standards (Kehar and Sastry 1948, Kehar and Mukerjee 1944) The biological value of proteins of different oilcakes was similar in the ruminant but varied widely in rats (Kehar 1945) Contrary to earlier findings (Ramiah 1939; Ray Sarkar and Sen 1939) muscular work has not been found to increase protein requirement, provided sufficient calories in the form of available carbohydrate or fat are provided (40) Lysine in sufficient quantity and in proper balance with ether amino acids must be provided for optimum milk production (Morris and Ray 1939). The high sulphur content of usar grass hay was found to interfere with nitrogen metabolism

Mineral Metabolism (Major elements)

A modified method for the estimation of major minerals in biological materials has been developed (Sen *et al.* 1942). The mineral content of a large number of grains, oilcakes, grasses, legumes and tree leaves has been estimated and their availability to the animal determined (Brito-Mitnnayagam and Abraham 1950, Chet Ram and Ray 1943; Lander *et al.* 1944, Mathur *et al.* 1943; Pandisekere *et al.* 1948; Ray 1945; Talapatra *et al* 1942; 1949). Tree leaves were found to

have a high calcium content, but due to low phosphorus content both these minerals are poorly utilized from this source (Chet Ram and Ray 1943; Momin *et. al.* 1947). The effect of the progress of maturity on the mineral content has been examined in grasses, tree leaves and fodder crops (Kehar, Ray and Agarwal 1947, Kehar and Talapatra 1947). The requirement of calcium and phosphorus by idle bullocks has been found to depend on the type of roughage used.

It was lowest with wheat straw as the roughage (Kehar and Qureshi 1944) and highest with rice straw (Chatterjee 1945, Chatterji and Mukerjee 1947; Sen, Ray and Ray Sarkar 1944) On feeding alkali-treated or water washed paddy straw, the requirement became the same as with wheat straw (Ray *et al* 1947; Sen *et al* 1942). Grasses in the high rainfall areas have a low calcium content, so that animals kept on such feeds show negative calcium balance (Kehar 1940; Talapatra *et al.* 1949). The calcium requirement has been found to vary directly with the body weight (Chatterji and Sarkar 1947). A regression equation for assessing calcium requirement under different dietary regimes has also been formulated (Chatterji 1940). Phytic acid present in cereal straw seems to interfere with calcium metabolism (Kehar 1950-51). Abortion in equines has been ascribed to calcium deficiency (Chatterji 1945; Rajagopalan 1939) The excessive sulphur and soda contents of usar grass hay are suspected to be responsible for the poor utilization of calcium and phosphorus from it.

Trace Elements

The importance of trace elements in the diet of livestock has been recognised only lately. Systematic work on the trace element content of food has been started only recently. The copper (Sahai and Kehar 1951) cobalt (Ahmed *et al.* 1939; Singh and Kehar 1948), manganese (Ray 1945; Parthasarathy and Mukerjee 1948; Sen 1942) and iron (Kehar and Murthy 1952) contents of grasses and common feeding stuff of India have been estimated. The maintenance requirement of these elements as well as the effect of their deficiency on the general metabolic behaviour of the animals have also been studied (Sahai and Kehar 1948). From these studies it has become apparent that trace element deficiency may be prevalent in parts of India. Cobalt deficiency appears to be correlated with the synthesis of B vitamins in the rumen of animals (Ray, S. N. *et al* 1947, 1948).

Some of the trace elements may be present in abnormally high amount in feed or drinking water, so as to produce pathological symptoms in the subject subsisting on such food or drink. One such element is fluorine and fluorine toxicity or fluorosis has been found to be prevalent in a number of places in India. Certain diseases which were supposed to be esteemalacia or rheumatic arthritis have been shown to be caused by the ingestion of excessive quantities of fluorine (Majumdar *et al.* 1943, Viswanathan 1944). A simultaneous low phosphorus intake aggravates the symptoms and precipitates the disease at an early date (Majumdar and Roy

1946, 1947). The histopathology of the disease has also been investigated (Pande 1944). The occurrence of a typical anaemia is the first recorded symptom This anaemia can be treated by administration of copper, cobalt and manganese salts (Sahai and Kehar 1948) Stoppage of fluorine ingestion or the simultaneous feeding of bone meal or aluminium salts brings about improvement in the conditions of animals (Majumdar and Roy 1946, Wadhwani 1945).

Vitamin Metabolism

The carotene content of a large number of feeds has been estimated (Kehar and Zubairy 1951, Sen and Talapatra 1943, Seshan and Sen 1942), and the stability of carotene in grasses and the destruction of this pre-vitamin during hay making and storage has been studied (Sen 1944, Seshan and Sen 1941, 1942). On dry feeds, animals are found to be in negative balance with respect to carotene Calves have been shown to synthesise vitamin A but the rate of synthesis can be augmented by several organic compounds (Ray 1942*b*). The requirement of vitamin C in ruminants increased in certain diseased condition (Ray 1942*a*).

Effect of Feed on Milk Composition

The fatty acid content of milk is found to decrease during inanition (Smith and Dastur 1938). The effect of heavy cotton seed feeding on the milk composition has been studied by several workers, the effect being apparent in the quantity and chemical characteristics of the milk fat (Patel *et al.* 1944, Patel 1944; Ray 1944). Green feeding also affects milk fat composition (Patel 1946, Ray Sarker and Sen 1939). The vitamin A content of milk is dependent on the carotene intake of the animal (Paul *et al* 1950, Ray Sarker 1948, Sen and Talapatra 1943, Seshan and Sen 1942) The vitamin A content of colostrum, was much higher than that of normal milk (Anantaramaiah *et al.* 1950, Ray Sarker 1948), but was much reduced in premilked animals. The vitamin C content of milk is independent of the nature of feed or of the breed of cattle (Seshan and Sen 1941, Ray *et al.* 1941), but is higher in buffalo milk (Khan and Bhatnagar 1945). Injection of thyroxine has been found to increase the milk fat content. The nutritive value of sub-adequate rations containing ghee has been found to be better than similar ration containing hydrogenated vegetable oil as the sole fat (Kehar and Chanda 1947*b*; Ray and Paul 1947).

Composition of Blood Tissues

The morphological and physiological constituents of the blood of healthy cattle, buffalo, goats, sheep, horses and lactating buffaloes have been determined (Mullick and Pal 1943; Pal *et al.* 1943, Kehar and Murthy 1945, 1948; Mac-Gregor 1943; Mukherji and Bhattacharyya 1948, Kehar, Singh and Rao 1940, Venkatarao *et al.* 1948). The composition has been found to change significantly from season to season (Pal *et al* 1943). When the diet is deficient in protein, vitamins, or minerals,

have a high calcium content, but due to low phosphorus content both these minerals are poorly utilized from this source (Chet Ram and Ray 1943; Momin *et. al.* 1947). The effect of the progress of maturity on the mineral content has been examined in grasses, tree leaves and fodder crops (Kehar, Ray and Agarwal 1947; Kehar and Talapatra 1947). The requirement of calcium and phosphorus by idle bullocks has been found to depend on the type of roughage used.

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The importance of trace elements in the diet of livestock has been recognised only lately. Systematic work on the trace element content of food has been started only recently The copper (Sahai and Kehar 1951) cobalt (Ahmed *et al.* 1939; Singh and Kehar 1948), manganese (Ray 1945, Parthasarathy and Mukerjee 1948, Sen 1942) and iron (Kehar and Murthy 1952) contents of grasses and common feeding stuff of India have been estimated. The maintenance requirement of these elements as well as the effect of their deficiency on the general metabolic behaviour of the animals have also been studied (Sahai and Kehar 1948). From these studies it has become apparent that trace element deficiency may be prevalent in parts of India. Cobalt deficiency appears to be correlated with the synthesis of B vitamins in the rumen of animals (Ray, S N. *et al.* 1947, 1948).

Some of the trace elements may be present in abnormally high amount in feed or drinking water, so as to produce pathological symptoms in the subject subsisting on such food or drink. One such element is fluorine and fluorine toxicity or fluorosis has been found to be prevalent in a number of places in India. Certain diseases which were supposed to be esteemalacia or rheumatic arthritis have been shown to be caused by the ingestion of excessive quantities of fluorine (Majumdar *et al.* 1943; Viswanathan 1944). A simultaneous low phosphorus intake aggravates the symptoms and precipitates the disease at an early date (Majumdar and Roy

linn have been found to produce non-fatal symptoms in bulls, sheep and goats (Kehar and Rao 1946).

Sheep and Goat Nutrition

A review on the role of nutrition in wool production has been published (Krishna 1939) Hand feeding of kids has been shown to induce better growth (Bhatia 1942) A comprehensive study of the feed requirement of Indian sheep has been undertaken.

Calf Nutrition

Experience regarding the effect of pre-milking on the health of calves has been found to differ in different farms In Delhi (Pan 1947) this practice was found innocuous while the results reported from Bangalore contradicted this (Kothawalla and Lazarus 1947) A schedule for raising calves on minimum quantity of milk has been given (Prasad 1945).

Pig Feeding

The essentials of pig feeding have been discussed (Shahi 1940)

IV(*b*) Dairy Animals

During the period under review, a number of publications bearing on the nutrition of dairy animals have appeared in India. These may be broadly classified under two heads (*i*) nutrition of young stock i e. calves, kids, etc., (*ii*) nutrition of adult animals, including heifers.

(*i*) *Nutrition of young stock*

The mortality rate among cow and buffalo calves is very high in the country and this is generally attributed to malnutrition and consequent low vitality. Yet, very little work has been done in India to remedy the situation. The little work done in this line is of a casual and sporadic nature Minett (1946) has studied the question of high mortality rate among calves in India and has suggested suitable remedies to lower this Millen (1946) has suggested methods of raising calves on a minimum amount of whole milk.

Bhatia (1942) has investigated the possibility of weaning kids at birth and hand-feeding them on warm milk. From these feeding trials he has concluded that the rate of growth of weaned kids was normal if not slightly better than that of the unweaned kids. Mortality among the weaned kids was also lower than that among the unweaned kids. Prabhu and Amble (1946) have made a statistical study of the effects of weaning kids and have come to the conclusion that weaning of kids has no untoward effect on the growth and performance of kids or on the goats themselves.

Prasad (1945) has given a schedule for raising calves on minimum quantities of whole milk. Whole milk was replaced by skim milk and concentrates from the third month onwards The quantity of skim milk was then reduced and concentrates increased gradually until from the seventh month the calves got only concentrates and no skim milk Under this scheme only 500 lb of whole milk was required for rearing a calf. Singh (1945) has enumerated the advantages and disadvantages of weaning calves and has described how best to look after them after weaning.

The suitability of an imported calf-starter-gruel for rearing calves has been studied by actual feeding trials at the Indian Dairy Research Institute. Gradually the whole milk was replaced completely by this gruel, but the results were not satisfactory either from the point of growth or mortality rates

Work on rearing calves on limited quantities of whole milk supplemented with skim milk, gruel, etc., has also been reported by the Indian Dairy Department. Substitution of 50 per cent of the whole milk ration by skim milk was found to be harmful to calves below 6 weeks of age, while it caused poorer growth in calves 6-13 weeks old But in the case of calves above three months, there was no harmful effect. Substitution of whole milk by soya emulsion in the feeding of calves has also been studied at Bangalore Although the digestibility coefficient of 50 50 soya emulsion and whole milk was lower than that of whole milk, the retention of calcium, phosphorus and nitrogen was quite comparable with that of whole milk The growth rate of calves fed on soya emulsion and whole milk was quite normal

Shark liver oil (vitamin A potency 1500 I.U /g) was fed as a supplement to young calves at the rate of 2 0 ml during the first month and 2 5 ml. during the second month at Bangalore There was no appreciable difference in the general condition and growth rate of the experimental calves as compared to the control group This is attributed to the comparatively high vitamin content of the feed the calves normally get on a farm It is expected that vitamin A supplement would give better results in the case of village calves which are at a low level of vitamin A intake.

Iodinated casein was fed to calves above six months at the rate of 2 g./100 lb. body weight over a period of 8 months at Bangalore. The rate of growth was less in the experimental animals and their nitrogen balance was also less satisfactory. It was concluded that iodinated casein does not exert any beneficial influence on the growth of calves.

(ii) *Nutrition of adult dairy animals including heifers*

By far the greater part of the work on the nutrition of the dairy animals in India during the period under review was carried out on adult animals with reference to the quantitative and qualitative aspects of the production of milk. Even here, one cannot say that the work has been carried out according to any preconceived

long range plan In fact, prior to this period there was very little appreciation of the nutritional requirement of the different species and different breeds of dairy animals in India. So also the information on the nutritive value of Indian feeds and fodders was of a scattered nature and limited in scope Sen (1952) brought together all the available information on Indian feeding stuffs in the form of a small booklet He has also dealt with the general principles of feeding livestock in general and the standard nutritional requirement for specific purposes like milk production, work, etc., with illustrations of the method of computing livestock ration.

Schneider *et al* (1939) have studied the digestibility data of one Sindhi and three cross-bred cows, all receiving the same ration and have come to the conclusion that there was little variation in the digestibility coefficients for the different animals.

Bal and Misra (1939) fed equal quantities of nutrients from different sources (*i*) groundnut cake, jowar, and turchuni and (*ii*) cottonseed, linseed cake and turchuni to two groups of buffaloes and found that the second ration was more favourable for milk production Morris and Ray (1939) have reported that excess protein in the ration decreased the milk yield

Das Gupta (1939) studied the effects of season, which, in this case was mostly the effect of feeds, on the carotene and vitamin A contents of the butter fat of cows in Bengal It was noticed that vitamin A and carotene increased when the animals were fed green fodder in winter.

The progress reports of the Cattle Feeding Research Scheme in Uttar Pradesh (Report of 1940, 1941) have given a number of interesting results on the effects of feeding berseem both as green and as hay on the growth of young animals and on the milk yield of milch animals. Replacement of 75 per cent and 50 per cent of the concentrates with berseem, as green and as hay respectively did not affect the milk yield of milch animals. In fact this kind of replacement of the concentrates was more economical There was no difference in the digestibility of the nutrients as between Hissar cows and Murrah buffaloes, and the milk yield on a fat corrected basis per unit of nutrients was higher in the case of Murrah buffaloes than of Hissar cows. Also, the average growth rate of the Murrah heifer was found to be higher than that of the Hissar heifers The same report also contains some results on the comparative feeding value of protein-rich grains, cakes and pulse by-products in relation to growth and milk production.

Kartha (1940) has studied the feeding conditions and management of Indian cattle all over the country and has come to the conclusion that almost 50 per cent increase could be obtained in their milk yield by balanced feeding and proper management. Ray (1940 *a*, *b*) has reviewed in a general way the standard of requirement for nutrients in the case of milch cattle and has also drawn up a scientific ration card for milch cattle for the use of dairymen.

Lander (1940) has reported some data on the metabolism of calcium and phosphorus in six heifers on a ration comprising oat-hay and groundnut cake. Calcium and phosphorus requirements of growing and milking animals have been determined by Ramiah (1940-41). Growing and pregnant cross-bred heifers (700 lb.) are found to need 40 g and 25 g of CaO and P_2O_5 respectively, while growing Kangayam heifers were found to need 35 g each of CaO and P_2O_5. Lactating cross-bred cows giving 15 lb. of milk were found to need 75 and 50 g respectively of CaO and P_2O_5.

Bal and Srivastava (1940) studied the periodic fluctuation in the carotene content of cow and buffalo butter fat caused by the variation in the intake of carotene. Doctor *et al* (1940) have reported that inclusion of grass in the ration increased the vitamin A and carotene in milk. Dastur and Smith (1939) have reported 40-50 per cent increase in fat output as a result of thyroxine administration. Das Gupta (*1943 a, b*) has published the detailed results of the berseem feeding trials, which were discussed earlier, and has advocated greater use of berseem in the feeding of all classes of livestock. Patel and Desai (1944) have described how a herd of 200 cows was being maintained on home-grown fodder from 153 acres of land without any decrease in the milk yield for a period of 10 years.

The possibility of feeding spineless cactus to dairy animals has been studied at the Indian Dairy Research Institute. Ghee residue and molasses were also fed to dairy animals at Bangalore in replacement of a portion of the concentrate mixture. While ghee residue did not affect the milk yield, molasses affected the yield adversely, probably due to the low intake of protein when molasses replaced part of the concentrates. Use of molasses in silage making was also studied with encouraging results at Bangalore (*Annu Rep., imp Dairy Dep* 1940, 1941).

Ukil (1942 *a*) has suggested that in wartime crop planning, adequate attention should be paid to feeds and fodder needs of cattle especially the dairy cow. He (1942 *b*) has also advocated the extensive cultivation of soya bean in India in order to alleviate the dietary deficiencies of both the human and animal population. Narayanan and Bhale Rao (1945) have discussed the possibility of utilising raintree pods as cattle feed. Dharmani *et al.* (1946) have studied the different aspects of feeding farm animals. Das Gupta (1946) has reported an increase in the milk yield of cattle when they were fed iodinated casein. Sikka (1947) and Williamson *et al.* (1947) have separately dealt with the food requirement of milch and other cattle in India and the latter has suggested ways of adjusting the agricultural practices to provide enough food for the cattle. Das Gupta (1947) and Ramswarup (1947) have made a survey of the feed and fodder requirements and resources of Uttar Pradesh and Delhi respectively.

Nanda (1942) has conducted feeding trials on dry cows at Hissar and has concluded that cotton seed was better for fattening than the same weight of a mixture of equal parts of oats, wheat bran and gram. Lander and Dharmani (1945)

fed fuzzy cotton seed to milch buffaloes without any detrimental effect on their general health and the quality and quantity of milk produced by them Patel *et al.* (1946) and Patel and Ray (1948) have studied the different aspects of feeding cotton seed to milch buffaloes on the output of milk and milk fat The milk yield increased in the third week by a maximum of 7 5 per cent but started declining in the 7th week Similarly there was a steep rise in the fat output after a week and this also gradually declined from the 3rd week On withdrawing green feed completely, the milk and fat yield declined considerably

Paul and Rangaswamy (1947) have studied the suitability of sugarcane tops for silage making and have reported encouraging results from feeding of the same to milch cattle. Millen (1947) has studied the economic aspects of cow keeping and has come to the conclusion that an animal producing 2,000 lb of milk per year pays its maintenance while a 3,000-pounder may yield a slight profit Compared to buffaloes, a good cow was always more profitable according to this author, but when compared to cows of the low production group, buffaloes were more profitable

Groundnut, cocoanut, cotton seed and sesame oils were fed to cows and buffaloes at the Indian Dairy Research Institute without any ill-effect on the health of the animals. The effect on the output of milk and milk fat was neither very marked, nor lasting, nor was it the same for the different oils. The composition of the butter fat was influenced by oil feeding Almost similar results were obtained when cows and buffaloes were fed cocoanut and groundnut vanaspati at the same Institute The suitability of brewer's yeast for cattle feeding was also examined at the Indian Dairy Research Institute but it was found to be without any special value Administration of nicotinic acid to Sindhi cows at the Indian Dairy Research Institute also did not produce any appreciable benefit (*Annu Rep imp Dairy Dep* 1944, *1945, 1946, 1947*).

Gupta (1948) has calculated the cost of making silage out of jowar at Karnal farm. Rajendra (1949) has studied the causes of the general deterioration of cattle on the west coast and has concluded that unbalanced rations are mainly responsible. Das Gupta *et al* (1949) has studied the feeding value of sugarcane including its effect on milk production, the concentrate could be cut down by 50 per cent Murari (1949) has advocated a realistic approach to the cattle problem in order to *maintain the present* stock in a healthy condition within the valuable feed resources of the country.

Seshan and Sen (1942) have studied the carotene metabolism of cows and bullocks at different levels of carotene intake and have shown that under ordinary farm conditions cows may secrete a fair amount of carotene and vitamin A in milk. With higher intake of carotene, carotene and vitamin A potency of milk increased. Sen and Rai Sarkar (1942) have also studied the carotene and vitamin A potency of cow's milk at different levels of carotene ingestion When a carotene-low ration was replaced by a carotene-high ration, the carotene and vitamin A of butter fat

increased considerably and *vice versa* Under heavy carotene feeding, the maximum contribution of carotene to vitamin A potency of butter fat was 35 per cent. The same authors (1943) have also reported that the fat, non-fat solid, total protein and ash contents of milk remained constant when a dry ration was replaced by intensive green feeding Ingestion of 1.5 — 3.0 million I U. of carotene per day produced a maximum potency of 10,000 — 11,000 I U. of vitamin A per pound of butter fat. Feeding 35 lb. of berseem or dub grass or 45 lb of mixed green grass or pasture was sufficient to produce this maximum potency. At this level only 30 per cent of the total carotene was digested and 0 09 per cent was recovered in milk Carotene accounted for 24 per cent of the vitamin A potency of the milk. Rai Sarkar (1945) has indicated the methods by which the vitamin A content of the milk could be increased

Patel and Ray (1945) have stated that the analytical constant of ghee were altered by replacing 50-100 per cent of the production ration by cottonseed irrespective of whether the animals were grazed or not Anantakrishnan *et al* (1947) have studied the effect of supplementary feeding of oils and vanaspathis up to a maximum of 1.5 lb. per head per day on the analytical constants and composition of the milk fat of cows and buffaloes Anantharamiah *et al.* (1950) have also studied the effect of varying the intake of carotene on the vitamin A potency of the milk of buffaloes.

Efficiency of utilisation of feeds of milk production by cows and buffaloes has been investigated at Bangalore. Cows were fed 100 per cent, 75 per cent and 50 per cent nutrients according to Morrison's standard with half the animals in each group getting guinea grass for fodder, and the other half Napier grass. Fifty per cent reduction in the nutrient intake affected the weight and milk yield of the animal whereas with 25 per cent reduction of Morrison's standard did not affect the milk yield much. The group receiving 100 per cent nutrients put on weight, this being more pronounced in the Napier grass group. No conclusive results were obtained on the comparative merits of the two grasses.

V. Parasitology

The great majority of the livestock in India is infected clinically or sub-clinically with parasites Acting insidiously as they do, parasites cause more than fifty per cent of the losses due to morbidity, stunted and retarded growth, poor productivity and mortality. There is scarcely a state in India where parasitic infections in one form or another do not constitute heavy economic burdens and cause severe annual and recurrent wastage of livestock wealth The experience in several foreign countries and in some parts of India suggest that the incidence of parasitic diseases, specially of helminths, will assume enormous proportions when the contemplated major schemes of irrigation are put in operation over large areas. For a considerable time there was a general lack of appreciation of the recurring heavy wastage of animal

wealth due to the depredations of parasites This has been due to the fact that the common symptoms of parasitic infection are not always sufficiently spectacular to attract attention or are masked by other infections However, during the period under review there has been a growing realisation of the economic importance of the control of parasitic infections, and considerable progress has been made in researches into the many-faceted problems of veterinary parasitology in India The salient achievements in the three main branches of parasitology, i e protozoology, entomology and helminthology, are briefly reviewed below

Protozoology

Since the publication in 1938 of 'Progress of Sciences in India', a considerable amount of work has been done in Veterinary protozoology in India This has been to a large extent due to the appointment of a Protozoologist at the Indian Veterinary Research Institute and Veterinary Disease Investigation Officers in the States, under *schemes sponsored by the Indian Council of Agricultural Research Protozoal infections in animals present diverse problems* and cover a much wider scope than in human medicine. Transmission of protozoa affecting the alimentary canal of the final host (amoebiasis, trichomoniasis, coccidiosis, etc) is brought about through the ingestion of infective material, while others are transmitted through vectors

Surra, produced by a haemoflagellate called *Trypanosoma evansi*, is the most important of the protozoal diseases of Indian domesticated animals A conservative estimate based on the data available for two years only shows that the monetary loss caused by deaths alone due to surra amounts to rupees one and a half lakhs annually, besides the much greater loss resulting from marked decrease in the working capacity of draught animals and in the milk yield of milch animals. Its occurrence in camels, horses, *mules, cattle, buffaloes, goats, elephants and dogs, etc.* has been reported by several workers Iyer (1948) observed some uncommon symptoms in bovine surra From biometric studies, Ray (1945) showed that specimens of *T evansi* from the horse and camel were smaller than those from cattle Mudaliar (1945) described as *T evansi var rayi* a small trepanosome from a buffalo *because it differed in certain morphological* and biological characters from *T evansi* Polymorphism has been noticed in one of the equine strains of *T evansi* maintained at the Indian Veterinary Research Institute Syngamy has been observed in this species in forms from a naturally infected mule

Iyer (1947) observed that in endemic areas acute surra in ponies occurred during the months of January to October, *the maximum number of animals being affected* during the fly season, i e. June to August Cases of surra have been successfully treated by a number of workers with tartaremetic in cattle and dog and with naganol or antrypol in camels and horses

Ray (1945) drew attention to the fact that symptomless, latently infected cases of cattle surra helped in the perpetuation *of the disease in India. He (1945, 1950)*

described allergic tests for detecting latently infected cases of trypanosomiasis in bovines, which act as carriers of the disease. Ray and Harbans (1948) have shown that pantothenic acid exerted an inhibitory action on the rate of multiplication of *T. evansi* in rats.

Crithidial forms of *T. theileri* of cattle was seen to occur in the peripheral blood of a calf while the gut contents of *Melophagus ovinus* revealed crithidia morphologically indistinguishable from the crithidial stages of *T. melophagium* of sheep and goats (*Annu Rep. Indian Vet. Res Inst.* 1939-40).

Leishmaniasis

Pande (1941) reported a case of cutaneous leishmaniasis in a bullock in Assam. The organism involved was suspected to be *Leishmania donovani*.

Trichomiasis

Enterohepatitis in a fowl due to *Trichomonas gallinae* was reported by Mohteda (1944). Rao (1946) reported high mortality in army pigeons.

Babesiasis in cattle

A single record of the occurrence of *Babesia bovis* in India was made by Idnani (1939) from a buffalo and Narasu (1939) recorded *B. bigemina* from the same species of host Ramanarayanan (1942), Rahman (1944), Prasad (1945), Hanumantha Rao (1945) and Salunke (1948) have recorded cases of babesiasis. *Babesia berbera* from buffaloes and *B argentina* from cattle have also been reported in India (*Annu Rep Indian vet Res Inst.*, 1938-39, 1939-40)

Ray (1938) showed that in the Indian strain of *B. bigemina* the situation of the Feulgen-positive nucleus conformed to the description of Nuttall and Graham-Smith (1908)

Ray and Raghavachari (1941) described a new species of Babesia, *B. foliata* from a sheep, *B motasi*, *B. sergenti* having been known to occur in this country for a long time *Babesia caballi* and *Nuttallia equi* are known to occur in horses in India Army authorities in India have reported successful treatment of equine piroplasmosis by Pirevan. Sheik Chingishah (1944) successfully treated a case of *N. equi* infection with two doses of antimony tartarate. Mudaliar, Achary and Alwar (1950) described a new species, *Babesia catti*, from a healthy cat.

The occurrence of *Aegyptionella pullorum* infection in poultry has been recorded from various parts of the country A consignment of ticks, *Argas persicus*, from Hyderabad (Dn) when fed on fowls immunised against spirochaetosis at I.V.R.I. transmitted *A. pullorum* into two birds out of four The salivary glands of these ticks showed the developmental stages of the parasite in their acini (Ann. Rept IVRI, 1949). Mohetda (1947) recorded a mortality ranging between 30 and 40% in fowls infected with *A. pullorum*.

Chetram (1940), Nagar (1942), Shetty (1940), Kapur (1943), Ray and Idnani (1943) and Narayanan (1947) have recorded cases of *Babesia gibsoni* infection in dogs and their treatment with various arsenicals Ray (1943) described a new genus *Pattonella* for *B. gibsoni* Swaminathan and Shortt (1937) showed that the infection was transmitted through the bite of the tick, *Haemaphysalis bispinosa.*

Theileriasis. In India *Theileria mutans* is very common amongst cattle but is not known to produce any harmful effects. Kulkarni (1941) however attributed calf mortality in a farm to *T. mutans* infection Ramanarayanan (1943) found Euflavine (May & Baker) useful against *Theileria (Babesia) mutans* infection in cattle, and Ramiah (1946) recorded cases of *T mutans* and *T. annulata* infections in buffaloes associated with high fever, congestion of the eyes with conjunctivae much swollen and mucopurulent discharge The fever however subsided leaving behind corneal opacity.

Ray (1950) has shown that the causative parasite is transmitted by a tick, *Hyalomma savignyi (aegyptium)*, and that the progeny of the infected tick are also capable of transmitting the infection but only in their adult stages.

Coccidiosis

In Indian livestock and poultry coccidiosis is very common, the common genera being *Eimera* and *Isospora.* Cases of canine coccidiosis due to species of *Isospora* have been reported by Mookerjee and Dass (1945) and Narasu (1948) Moberley (1947) described cases of calf coccidiosis treated successfully with 1 per cent solutions of copper sulphate given orally along with astringents and Biswal (1948) successfully treated coccidiosis in buffalo calves with an astringent mixture, sulphaguanidine and sulphamethazine. The last named drug gave cent per cent cure.

E. zurnii and *E. bovis* are commonly met with in Indian cattle On two occasions the occurrence of *E cylindrida* and *E thianetti* was noted in hill bulls at Mukteswar (*Annu. Rep. Indian Vet Res Inst.* 1937-38; 48-49).

Indian sheep and goats commonly harbour *Eimeria arloingi*, *E faurei*, *E. intricata*, *E parva*, *E. pallida* and *E. ninakohlyakimovi*, and *E crandallis.* Six species of Eimeria, viz. *E. tenella*, *E. acervulina*, *E. mitis*, *E. maxima*, *E. necatrix* and *E. praecox* have so far been reported along with a species of Wenyonella, *W. gallinae* Ray (1945). Of these species, *E tenella* is highly pathogenic to birds of tender age causing great economic loss to poultry farmers (Ray 1947).

E steidae produces heavy mortality in young rabbits. Fatal cases invariably show liver coccidia, *E. steidae*, although concurrent infection with *E. perforans*, *E. neoleporis*, *E. magna*, *E. exigna*, *E. irresidua* are not uncommon.

Occurrence of *E meleagrimitis* in turkey chicks was recorded from India in 1948 (*Annu. Rep. Indian vet. Res. Inst.* 1948).

Haemoproteus

Aiyar and Garudachar (1939) recorded species of *Haemoproteus* in the blood of peacocks. Rao (1946) in his studies on trichomoniasis in pigeons reported the occurrence of *Haemoproteus* columbae infection also in them.

Hepatozoon. Ramamurthy (1939), Rehimuddin (1945) and Seetharamamurthy (1946) have recorded cases of *Hepatozoon canis* infection in dog and its treatment with acetylarson

Entamoeba. Entamoeba was encountered in the faeces of Indian goats and sheep. The one from the goat was morphologically indistinguishable from *E debliecki* while the one from the sheep still remains to be identified.

A species of *Pentatrichomonas* was reported to have produced deaths in ducks in Bombay.

A few cases of giardiasis in dogs were encountered in India, the organisms being morphologically indistinguishable from *Giardia canis.* Experiments carried out at the I.V R I to determine the infectivity of *C. lamblia* (human form) to pariah dogs have negative results. This confirms the observation of Hegner, 1922. Incidentally it may be mentioned that a few cases of giardiasis in human beings were successfully treated with therapeutic doses of quinacrine (Pant and Ray 1942).

Encephalotozoan Ray and Raghavachari (1941) recorded for the first time in India the occurrence of *Encephalotozoon cuniculi* from the kidney of rabbit

Toxoplasmosis. Occurrence of *Toxoplasma canis* in a pure-bred spaniel which had succumbed to *Pattonella gibsoni* infection was reported by Ray and Raghavachari (1941).

Bartonellosis. Bartonellosis due to *Bartonella canis* infection in dogs was recorded by Ray and Idnani (1940).

Rickettsiosis The occurrence of a Rickettsia morphologically indistinguishable from *R ovina* was for the first time encountered at I.V.R.I. in the blood of 3 sheep.

R canis infection in dogs have also been encountered in this country (*Annu. Rep. Indian vet. Res. Inst.* 1943-44; Mudaliar 1944).

Sarcocystis. Sen (1949) reported the occurrence of spores of Sarcocysts in large numbers in heart blood smears of 12 cattle and one sheep.

Ray and Singh (1949) reported *Trichomonas thukunei* from the caecum of laboratory guineapigs (*Cavia cutleri* Bennet). The occurrence of a 'Pelta' was for the first time observed in this trichomonas.

The caecal contents also showed *Trichomonas cavie.* The intestines of *Leiolopisma himalayanem* was infected with a species of *Eimeria* which was characterized by supra-epithelial development in all its stages.

Quite a number of protozoal parasites have been recorded from wild animals and birds (Aiyar and Garudachar 1939, Sapre 1944; Lakshmanaraju and Swaminathan 1947; Ray and Sapre 1945).

Besides the allergic test for detecting latency of trypanosomiasis in cattle evolved by Ray (1945, 1950); he (1944) described a method for rapid staining of intestinal flagellates Ray and Bhattacharya (1948) have reported a useful method for cutting sections of ticks.

Entomology

Arthropods (insects, ticks and mites) are injurious to animals both as direct pests as well as vectors of a number of diseases. Veterinary entomology is concerned with the study of these arthropods, their predators and parasites, the transmission of animal diseases by them and the measures for their control A considerable proportion of the mortality among the livestock in India, like that in many other countries, is due to diseases transmitted by arthropods As pests they affect the growth, vigour and productive capacity of animals adversely. It is obvious that in a tropical country like India, the arthropod damage to livestock is enormous and the annual loss of money therefore must amount to many crores.

Investigations on Veterinary entomology carried out in this country during the period under review were mostly concerned with studies on (1) the transmission of animal diseases through the agency of arthropod vectors, (2) survey of distribution and seasonal incidence of arthropods of veterinary importance and their parasites and predators and (3) the effect of some insecticides in the control of these arthropods.

Transmission of animal diseases through the agency of arthropod vectors

Studies on the transmission of some of the animal diseases like surra, anthrax, rinderpest, theileriasis, fowl spirochaetosis and haemorrhagic septicaemia through the agency of arthropods were one of the main lines of investigations during this period. *Haematopinus tuberculatus* and Musca spp (mostly *nebulo*) have been shown experimentally to be the vectors of surra, a fatal disease of horse, mule, camel, etc. *Pasteurella avicida*, the causal organism of chicken cholera was shown to be transmissible by the fowl tick *Argas persicus*. The mechanism of the tick transmission of fowl spirochaetosis was thoroughly investigated During this period the tick transmission of *Spirochaeta cobayae* the causal organism of relapsing fever of guineapigs was established. *Ctenocephalis felis* was shown to transmit haemorrhagic septicaemia from diseased to healthy rabbits. It was proved that *Musca domestica*, *Musca nebulo*, *Ctenocephalis felis* and *Hyalomma savignyi* can transmit anthrax from diseased to healthy animals. The flies *Tabanus orientis* and *Stomoxys calcitrans* were shown to be capable of transmitting rinderpest disease. Studies on the arthropod transmission of fowl malaria and paddy bird malaria were also carried out during this period.

Survey of distribution and seasonal incidence of arthropods of Veterinary importance

During this period quite a considerable amount of survey work on the distribution and seasonal incidence of several species of arthropods having veterinary importance was carried out by the staff of the Entomology section of the Indian Veterinary Research Institute, and the Disease Investigation Officers of Civil Veterinary Departments of various States It had been found that several important pests of livestock in India are distributed over a large area in the country that had been previously believed to be free from them. *Hypoderma lineatum* and *H. crossii*, the ox and goat warble flies respectively and several species of cattle ticks and horse-flies have been mapped to show their distribution in the country. The seasonal incidence of some of the arthropods of Veterinary importance in and around Izatnagar had been studied. Studies on the life histoy and bionomics of *Hunterellus hookeri*, a parasite of ticks and *Telenomus* spp. a parasite of tabanus fly eggs were in progress.

Studies on the effect of some insecticides in the control of arthropods of veterinary importance

Experiments to study the effect of several insecticides such as DDT, BHC, derris, nicotine, pyrethrum, etc, in controlling a number of species of insects, mites and ticks injurious to domestic animals have been carried out. Though these experiments were of a preliminary nature, several of them yielded interesting and useful results The warble-fly could be successfully controlled in the field by singeing the hairs on the legs of cattle during the egg-laying season of the fly Some proprietary preparations of insecticides namely Brizk, Pyrocolloid, Pyro dust 1500, Pip, Gamatox paste dip, Anofilum, Kildem, Pyrentol D20, Neocid and No 1050-Geigy have been tested on a number of species of arthropods attacking domestic animals. Some of these chemicals were found to be quite effective against some species of flies, ticks and lice

HELMINTHOLOGY

Veterinary helminthology aims at the conservation of animal health by attempting the control of parasitic worms of livestock. Helminth parasites affecting domesticated animals are legion, and differ widely in their structure, biology, habits and injuriousness, and in consequence the methods for the treatment and control of different helminthci infections differ considerably. Barring a small group of trematodes, they are internal parasites and no part of the body of the host is free from their attack. They cause damage to the host in a variety of ways: depriving the host of its feed; feeding on the tissues of the host; mechanically causing irritation, injury, obstruction or pressure, and causing abrasions through which secondary infection may take place; by formins nodules, tumours or sores and elaboration of substances injurious to the host. Their economic significance may be

realized from their constant association with a number of disease conditions, the retarded growth of the affected animals, the chronic ill-health, reduced usefulness, pronounced morbidity and high mortality among livestock which cause recurrent losses in wide spread and numerous endemic areas in the country.

The period under review (1938-1950) has witnessed considerable progress in researches on the various aspects of the problem of control of helminth parasites of animals of economic importance This has been possible mainly due to the devotion and hard work of the officers of the Indian Veterinary Research Institute and of some Veterinary Colleges and the staff employed under schemes financed by the Indian Council of Agricultural Research

A knowledge of the helminth parasites of domestic animals is necessary before priority for investigations leading to the elimination of losses due to them can be determined Prior to 1938, work on the occurrence of these parasites, their morphology and systematics had largely held the field. During the years under review extensive surveys into the helminth parasites of animals of economic importance were carried out by Moghe (1945) and Srivastava (1945) Several workers have recorded the occurrence of a large number of species of new or unrecorded helminths and have described their morphology and systematic identification. A number of parasites affecting food fishes and wild animals have also been studied either for their economic importance or because they may act as 'carriers' of parasites of domesticated animals and man

Helminth parasites do not multiply within the body of their final hosts and every adult worm found inside an animal must have entered as infective larva from outside During the developmental stages outside the host they are not immediately infective and a definite period of time and favourable conditions are necessary for their development to the infective stage In the life cycles of a vast number of these parasites, intermediate hosts are intercalated. Thus a sure method for the prevention of infection lies in interrupting the life cycle at some convenient stage However, the life cycles of worms differ widely and a detailed knowledge of them is necessary for formulating effective and rational control measures. The life histories of nearly two dozen species of worms have been worked out partially or completely. Srivastava (1938) worked out the life history of *Cotylophoron Cotylophorum* an amphistomatous parasite of ruminants and described its pathogenicity. The life history of a common tapeworm *Mesocestoides lineatus* of Indian dogs and cats was also elucidated by him (1939). Vaidyanathan (1941) infected experimentally a calf with *Fischoederius elongatus* with cercariae indicae XXIX sewal. A detailed study of the life history of the bovine nodular worm, *Oesophagostomum radiatum*, was made by Anantaraman (1942). The intermediate hosts of several trematode parasites *Dicrocoelium dendriticum*, *Paramphistomum explanatum*, *Gastrothylax crumenifer*, *Fasciola hepatica* and *F. gigantica* of ruminants were discovered by Srivastava (1944 a-f). The intermediate host of a common

lungworm in sheep and goats at Mukteswar was discovered by Bhalerao and Kapur (1944) and Bhalerao (1945). The latter worker (1947) reported a species of landsnail acting as intermediate host of *D. dendriticum*. The life history of a common whipworm — *Trichuris ovis* — of ruminants was studied by Deo (1947 *a* and *b*). Peter and Mudaliar (1948) described a new cercaria and experimentally showed it to be the larva of *Gastrodiscus secundus*. The life history of an acanthocephala — *Moniliformis moniliformis* — was studied in detail by Sita (1949). Pre-natal infection of *Ascaris vitulorum* in buffalo calves was reported by Vidyanathan (1949). Basir (1950) studied the development of a nematode of sheep, *Strongyloides papillosus*, and Sinha (1950) of *Cotylophoron cotylophorum*, an amphistome of ruminants The life history and biology of two species of tapeworms of fowl have been studied by Dutt and Sinha (1950) and Dutt, Sinha and Mehra (1950).

Helminthic diseases are characterised by widely different manifestations. During the period under review several interesting conditions have been proved to be of helminthic origin. Srivastava (1938) established experimentally that animals infested with immature forms of *Cotylophoron Cotylophorum* became dull, weak and anaemic and showed general unthriftiness and persistent foetid diarrhoea. The duodenum and intestine contained haemorrhagic fluid and the intestinal mucosa was markedly thickened and necrotic The faeces contained numerous immature amphistomes. Recovery followed the migration of the young parasites to the rumen when they were non-pathogenic Outbreaks and symptoms of amphistomiasis have also been described by Bawa (1939) and Mudaliar (1944, 1945). Srivastava (1947) studied the pathogenicity of the pouched amphistome — *Gastrothylax crumenifer* — of ruminants and investigated the factors governing it Prosthogonimiasis in Indian poultry was for the first time recorded by Srivastava (1938) and the causative parasite described Datta (1939) studied and discussed in detail microfilarial pityriasis in equines Though the exact identity of the associated microfilaria has not been determined, the discovery by Srivastava (1938) of *Onchocerca cervicalis* in horses in India is suggestive Iyer (1938) and Ayyar (1944) reported the occurrence of verminous ophthalmia in equines and canines respectively. Datta (1938), Srivastava (1938, 1939), Srinivasan (1939) and Rao (1941) have described a form of generalised microfilarial dermatitis in bovines. An unusual case of microfilarial granuloma in the prepuce of a bullock has been described by Naithani and Narayanan (1940) The occurrence of verminous pneumonia was recorded for the first time by Srivastava (1940). The occurrence and symptoms of nasal granuloma in goats was recorded by Malkani and Prasad (1941). Stephanofilariasis in buffaloes in Assam was reported by Gopalakrishnan (1949).

Curative treatments of animals infected with helminths is at present often not feasible as most of the stock-owners are poor, the value of the livestock is low,

and the parasites may be located in inaccessible tissues and organs and immunity is absent in treated animals. Cheap and effective anthelminthics easy of administration, and of sound and rational control measures against most of the helminth parasites remain to be developed. Though much valuable work has been done and a lot of ground covered, the entire field of mass application of measures and country-wide campaign for control of helminth parasites are yet to be evolved and implemented.

VI. Poultry Research

Although some improvement in the development and organisation of the poultry industry had been effected in India prior to 1939, the industry remained unimportant mainly because of the lack of appreciation of the value of eggs and poultry as human food On the recommendations of the Royal Commission on Agriculture 1927, a Central Poultry Research Division was established at the Indian Veterinary Research Institute, Izatnagar in 1939 At present this is the only station in the country where fundamental research on poultry science is being carried out. The functions of the Division are both scientific and practical, and the main laboratory buildings provide ample facilities for research into breeding nutrition, physiology, technology and pathology Attached to the Division are also two experimental poultry farms where methods of practical poultry breeding and maintenance are evolved and demonstrated Some of the outstanding results of research on poultry science are listed below

Evolution of Indian breed for high production — In India the village hen is notorious for its small size, low egg production and the small size of its egg By a process of culling and selective breeding for economic qualities over ten generations, an improved strain of Indian fowl has been evolved whose average annual egg production is of the order of 150 while individual hens have laid up to 237 eggs as compared to the village fowl whose average annual egg production is only 53. The strain evolved is good for both egg production and meat

Grading — The grading up of low-production stock of Indian fowls by cocks of foreign breeds such as White Leghorns, Rhode Island Reds and Barred Plymouth Rocks has been found to be the quickest method of improving an ordinary farm flock in regard to egg production, egg size and the elimination of broodiness. The eggs produced by the graded hens are significantly large and the egg production is nearly doubled. Extensive grading operations in the States are in progress.

Pure breeds — At the Indian Veterinary Research Institute farm-pedigreed strains of exotic breeds of poultry such as White Leghorns, Rhode Island Reds, Black Minorcas and Barred Plymouth Rocks have been acclimatised, bred and multiplied. Foundation stocks of these breeds have been supplied to the various State farms and private poultry breeders every year.

Sex-linkage — In the production of table fowls, sexing of day-old chicks to eliminate the females adds to the economy. Suitable crosses using Barred Polymouth

Rocks imported from U.S.A. and a variety of Indian fowls have been produced in which the sexes of the baby chicks can be accurately determined. In the table poultry trade this method will help to reduce the cost of production significantly.

Village Poultry Keeping — Studies on egg production in villages have revealed that the high rate of production of foreign breeds of poultry is not maintained unless the birds are adequately fed. Some form of cheap animal protein supplement in the ration has been suggested.

Inheritance — Experiments on the inheritance of morphological characters, such as smut (slaty) under colour in Rhode Island Red fowls, and feathers on the shanks and feet of White Leghorns have revealed that these characters which are considered as breeding defects are not associated with egg laying qualities. The results have practical importance as valuable birds which are good producers are being killed for the table by fanciers out of ignorance

The various factors responsible for the determination of fecundity of birds, early sexual maturity, high rate of production, non-broodiness, lack of winter pause and persistency of production have been studied under Indian conditions.

Nutritive value of egg — Investigations have been carried out to determine, (*a*) the number of eggs that should be added to a Bengali village diet to produce optimum growth in rats, (*b*) the supplementary value of eggs and the various common pulses in the village diet From the nutritional standpoint it was observed that with eggs as the sole supplement to satisfy the necessary requirements for growth and maintenance in children, it would be necessary to consume 5 eggs daily, a ration which would be much too costly for the majority of the population. Substitution of various levels of pulses for the egg supplements demonstrated that on both nutritional and economic considerations, a Bengali diet in conjunction with a daily supplement of one egg and soyabeans (66 g) or green gram (130 g) or gram (157 g) proved very satisfactory.

Protein Supplement — Controlled experiments on the value of different protein supplements in poultry rations revealed that (*a*) a cereal ration plus one per cent common salt in conjunction with liberal amounts of green food and limestone, is unsatisfactory both for growth and egg production; (*b*) separated milk is a very valuable protein supplement in poultry rations; (*c*) meat offal as protein supplement gave almost as good results as separated milk As meat offal is usually obtainable at low cost, it should be used more freely in poultry rations, (*d*) soyabean meal and groundnut cake both proved inferior to separated milk or meat offal as protein supplements.

Digestibility Co-efficients and Biological values — A method for determining the digestibility co-efficients and biological values of proteins in poultry rations has been standardised. The method has proved very useful for the determination

of the nutritive value of common Indian feeds for the formulation of economic rations for poultry.

Feeding of Cow manure — The poultry feed represents the largest single item of expense in the production of poultry and eggs, accounting for approximately 60 per cent of the total cost of production. In order to evolve a method for economical production of meat and eggs, investigations have been carried out on the use of cow manure as a supplement to an all-plant poultry ration Cow manure contains both the growth promoting factors and the androgenic principles It has been demonstrated that the egg production is decreased by 10 per cent as a result of the inclusion of 8 per cent dried cow manure of high androgenic potency in a good practical all-mash diet, but the processed cow manure in which the androgenic potency had been destroyed by drying at 80°C for 24 hours, or removed by treatment with water, exerted no adverse effect on egg production The inclusion of the processed cow manure in a diet containing no animal protein supplements has been shown to improve hatchability significantly.

Waste Foods — Studies on the effect of the addition of mango seed kernel and jaman seed meal in a simplified poultry ration for egg production have revealed that mango seed kernel, which is generally grown away as waste material, can be utilised for replacing as much as 20 per cent yellow maize meal in the laying ration with beneficial results Tht inclusion of jaman seed meal on the other hand, showed a large depressing effect on egg production Further experiments with growing chickens demonstrated that both mango seed kernel and jaman seed meal were equally efficient in replacing as much as 20 per cent yellow maize meal in the growing ration. The factor present in the jaman seed meal which has a depressing effect on egg production does not therefore appear to have any deleterious effect on the growth of chickens

Vitamin A deficiency — During the hot summer months mortality in all classes of poultry due to paucity of green supplements containing vitamin A in the rations often assumes serious proportions, particularly in specialised farms Experiments done on this problem have shown that the addition of small quantities of Shark Liver Oil in poultry rations as supplement in place of greens greatly helps to cut down losses among fowls due to deficiency diseases.

Egg spoilage — During most parts of the year, it is estimated that over a third of the country's output of eggs are lost due to embryo development, staling, breakages, moulds, bacterial contaminations etc Methods have been elaborated to minimise these losses, e g., defertilisation, lime sealing and oil dipping

Defertilisation is carried out by dipping eggs in water at 130°F for 15-30 minutes and then storing at low temperatures. This process prevents embryo development, stabilises the quality of albumen and improves the keeping quality of the eggs.

Lime sealing is usually done by dipping eggs in lime water and storing at low temperature. This prevents staling for several weeks.

In the case of oil dipping the eggs are dipped in oils, such as, mineral oil, or cocoanut oil and then stored at low temperature.

All these methods have great potentialities and are being extended to large cities by opening suitable egg grading stations under expert supervision.

Canning — In canning chickens, the use of lacquered cans is preferred, as plain tin cans develop dark spots on storage due to the action of the sulphur of the meat on the iron of the can. Investigations have also shown that the addition of common salt promotes the development of metallic taste even in the contents of the lacquered cans, especially when the lacquering is not perfect. The addition of salt is, therefore, not recommended in canning chickens.

The determination of the percentage of edible flesh in the Rhode Island Red White Leghorn and Desi (indigenous) cockerels at 8, 12, 16, 20 and 24 weeks of age respectively revealed that the percentage of total edible material was highest in the Desi and lowest in the White Leghorns at all stages of growth.

POULTRY DISEASES

(1) *Ranikhet (or Newcastle) Disease*

This disease is second to none in importance and the chick embryo vaccine for the control of the disease, first elaborated in this country, is now recognised as a biological one, and this has enabled successful poultry keeping by the villagers The vaccine is cheap, safe and the protection conferred by it lasts for the useful life span The vaccination has no adverse effect on either growth rate or production. Vaccine depots have been organised in some of the States.

(2) *Salmonellosis*

Salmonella infection of poultry is highly destructive to baby chick trade in all countries where poultry farming is a specialised occupation. India enjoyed freedom from this disease until recently Since 1949 outbreaks are being recorded in widely separated areas, but confined to specialised farms, causing chick mortality, reduced egg production and in some cases deaths among matured stock. In each case the spread of the disease has been controlled and the infection more or less eradicated by improved blood tests.

(3) *Fowl Cholera*

The existence of carriers responsible for perpetuation of the disease from one season to another has been brought to light. Work on the preparation of a more satisfactory vaccine has been in progress.

(4) *Avian Leucosis Complex*

This is a disease almost new to India though its incidence in widely separated areas has now been recorded. The casual agents have been shown to be a filterable fraction (virus) capable of being experimentally transmitted to susceptible birds. Valuable laying fowls are sometimes the victims of this malady

(5) *Fowl pox*

In recent years numerous reports on the incidence of fowl pox have been received, the mortality being very severe in chicks An English strain of pigeon pox virus has been used as a vaccine and the satisfactory age for vaccination has been found to be six weeks and above.

(6) *Avian Malaria*

The first field case of *Plasmodium gallinaceum* was recorded in India in 1949. The disease has been reproduced experimentally by blood inoculations and the chemotherapy and various other aspects of the infection have been under investigation.

In 1919 the U P. Government started at Lucknow, the U P Poultry Association which is carrying out very useful developmental work Another old Institution in the U.P. is the Mission Poultry Farm at Etah which was started in 1912 and now has a number of branch farms.

Various other provincial centres have carried out investigations on breeds such as White Leghorn and Rhode Island Red, occasionally Minorca and Cross breeds. Model Poultry Farms have been set up in most of the States of the Union which help to disseminate improved stock to the public

VII Animal Breeding and Genetics

The history of animal breeding work in India prior to 1938 has been reviewed by Ware (Prasad 1938) That period marked almost the first attempt at taking up organised livestock breeding activity in this country in recent times. Many breeding projects were taken up although they were extremely inadequate considering the variety and the vastness of the livestock population and the diversity of the problems. Many new farms came into being. Large scale campaigns for castration of scrub bulls, and dissemination of germ plasm of superior sires were initiated A good foundation for animal breeding research was indeed laid in which the contribution of the Indian (then Imperial) Council of Agricultural Research, which came into being during that period, is praiseworthy.

However, the initiation of animal genetics research and livestock breeding acti-

vities on sound scientific lines really belongs to the post-1938 period. The first research organization in animal genetics in the country came into existence with the establishment of the Animal Genetics Division of the Indian Veterinary Research Institute in 1944 This was followed by the creation of the post of Animal Geneticist at the Livestock Farm, Hissar, under an Indian Council of Agricultural Research scheme, and a similar one by the Uttar Pradesh Government. By the end of the period under review, many of the States had followed suit. During the present period a large number of projects on sound scientific lines were taken up at the Central Research Institutes and in the various States, on different aspects of animal breeding science including the breeding of better livestock. The Indian Council of Agricultural Research sponsored a large number of schemes in this direction

The magnitude of the farm animal industries in India may well be imagined from the population figures for the different kinds of our livestock and their produce as estimated by the Marketing Section of the Ministry of Agriculture, Government of India (1950) In the year 1950, the 20 breeds of the *Bos indicus* species totalled 5,67,86,370 adult breeding and non-breeding males, 4,46,02,904 breeding females and 3,85,81,800 young stock. These figures include the few herds that we have of the Sahiwal, Red Sindhi and Tharparkar breeds of cattle, the habitats of which have all gone to West Pakistan since the partition of the country The 10 breeds of the *Bos bubalus* species totalled 58,12,651 males, 2,04,08,183 females and 1,53,05,039 young stock The 16 breeds of our goats totalled 3,89,08,418 heads, and there were 37,12,361 swines in the country. The country also abounds in horses, camels, donkeys, etc. etc. The above animals produced 5,93,892 tons of bone, 2,05,78,042 hides, 3,70,35,257 skins, 6,58,346 maunds of wool, and 48,36,00,577 maunds of milk some of which was used for producing 1,12,96,481 maunds of ghee Immense as the size of our animal industries may appear to be, they are inefficient and production falls short of the needs of the nation In order to improve this vast livestock population, the effort of a large band of workers fitted with modern tools and knowledge will be required. All that has been done so far only touches the fringe of the problem. Farm animal breeding projects are time-consuming and costly, so that much more intensive work will be necessary.

The work done during the period under review is briefly outlined below:

Cattle Breeding

Selective Breeding

The systematic work conducted with various breeds of cattle substantiated the belief held previously that some of the well-defined Indian breeds had good potentiality for being developed for higher milk production. Encouraged by the

results of selective breeding work done on various farms, the general policy for cattle improvement in the country during this period has been :

(i) to improve the distinct breeds of cattle by selective breeding, wherever such breeds exist;

(ii) to grade up the non-descript cattle in undeveloped areas by the use of good quality bulls of the distinct breeds considered suitable for the area.

By continued selective breeding and controlled feeding and management, the average milk yield of Sahiwal cows at the Lyallpur Agricultural College rose to 6,000 lbs. per year in 1946 with individuals exceeding 10,000 lbs as against the 2,000 lbs. yield of the foundation herd in 1913 Yields of over 12,000 lbs milk in 305 days have been likewise achieved for the Sahiwal herds at the Ferozepur Military Dairy Farm and at the Indian Agricultural Research Institute. High milk yielding cows of the Red Sindhi breed have been produced at the Indian Dairy Research Institute, Bangalore, Agricultural Institute, Allahabad, and the Livestock Farm, Hosur At Hosur, lactations of over 10,000 lbs have been recorded Similarly the Tharparkar herds of Patna and Karnal have done well. Some of the Karnal cows have exceeded 10,000 lbs milk in 305 days Lactations of over 6,000 lbs have been obtained in the Gir herd at Bangalore

Remarkable achievements in the field of selective breeding during this period have also been reported with the Kankrej herd which has given lactation yields of over 8,000 lbs. at the Agricultural Institute of Anand, and with the Kangayam herd at Pallayakottai which has given lactation yields of over 4,000 lbs. These two breeds were formerly considered to be purely draft animals with no potentiality for milk. Work with the Gaolao breed at Wardha has likewise shown it to be capable of better milking capacity than was thought hitherto. The Hariana breed from the Karnal Farm was transferred to the Indian Veterinary Research Institute at Izatnagar during the period under review, and planned systematic work based on progeny testing of bulls has been taken up with this herd with a view to improving the milking capacity of the breed without deterioration in its draught qualities Lactations of 6,000 to 7,000 lbs. milk have been recorded in this herd Selective breeding has been taken up with these and other well-defined breeds in the various States in over 140 Government and private farms and over 3,000 gaushallas with promising results. A good fillip has been given to this system of breeding by the initiation and establishment of the Central Herd Books which have been gradually made open to more and more well-defined breeds. A minimum production of 3,000 lbs. milk in 305 days in the case of Sahiwal cows, 2,500 lbs. lbs. for Red Sindhi and Tharparkar cows, 2,000 lbs for cows of Gir, Hariana and Kankrej breeds, and 1,500 lbs. for Kangayam and Ongole breeds, makes a cow or its offspring, eligible for admission to the Herd Book of the relevant breed and enhances its reputation and value.

Cross-breeding

Cross-breeding between *Bos indicus* and *Bos taurus* did not find much favour during the period. Experience gained at the Military Dairy Farms showed that the higher grades of cross-bred animals had commercial possibilities but were not desirable as general stock in view of their high susceptibility to tropical diseases and lack of adaptability to the climate. In fat, except for commercial exploitation and for researches, cross-breeding almost became taboo. One experiment of Indo-European cross-breeding conducted at the Allahabad Agricultural Institute is worth mentioning Reciprocal crosses have been made between Red Sindhi and Jersey. The trend of performance of the small numbers obtained for the various grades seems to indicate that a genotype combining a little less than 3/4th Jersey inheritance with the Red Sindhi may help in evolving a useful Indo-European dairy breed for Indian conditions

Evolving new Indian breeds

A large experimental cattle breeding station was opened by the Central Government in 1947 with a view to explore the possibilities of evolving a new high milk-producing dual purpose breed of medium size by crossing the Indian dairy breeds, namely, Sahiwal, Red Sindhi and Tharparkar with some good draught breeds, namely, Nimari, Malvi and Gaolao Work was started on sound scientific lines, but the project unfortunately had to be closed in its infancy in 1951 due to financial stringency.

Grading up

The grading of nondescript cattle in under-developed areas with good sires of well-defined breeds received much impetus during this period and progress has been greatly accelerated by the use of the artificial insemination technique.

Use of milking cows for work

Work has been taken up at the Indian Dairy Research Institute, Bangalore, under a scheme sponsored by the Indian Council of Agricultural Research to find out the possibilities and scope of using milking cows of the Red Sindhi breed for work. The effect of work on pregnancy, lactation and health is being studied. The scheme envisages taking up of similar work on Hariana and Tharparkar breeds at the Indian Veterinary Research Institute, Izatnagar, and at the Karnal Farm respectively.

Buffalo breeding

The importance of buffaloes in the dairy industry of India is well recognised.

Of the world's total buffalo population of 76 million heads, more than 40 million are in India The average annual milk yield per buffalo is 1,100 lbs. as against 413 lbs given by an Indian cow. Buffalo milk is besides $1\frac{1}{2}$ times as rich in butter fat content as Indian cow's milk which makes it more valuable for butter and ghee production. Buffaloes are also better utilizers of coarse fodder than cows Being of greater economic value, buffaloes are naturally better looked after than our cows. Experience gained at the Government farms has shown that by better feeding and management, the production of village cows may be increased by 50 per cent, whereas that of buffaloes may be increased only by 15 per cent It must be mentioned however that in view of the early maturity of the cow, and greater usefulness of its male calves as draft animals, this species has greater overall utility Records of the Military Dairy Farms where both the species are maintained, indicate that when fodder is expensive, even the unimproved Indian cow produces milk and butter fat at cheaper rates than the buffalo Though the buffalo is much better developed than the Indian cow, attempts are in progress at the various Government and private farms to improve the buffalo's production capacity and efficiency by selective breeding within the well-defined breeds Elsewhere grading of nondescript buffalo stock with sires of well-defined breeds is widely adopted, for which purpose the Murrah breed is by far the most popular Murrah has also found a place in the herd book system, the qualifying limit being a minimum of 3,000 lbs milk in 305 days.

Goat Breeding

The goat is the poor man's dairy animal in India It is also a major source of animal protein in the dietary of the vast non-vegetarian population of the country. To improve productivity in goats, several goat breeding schemes were initiated by the Indian Council of Agricultural Research in the various States Selective breeding and/or grading was taken up in the Punjab with the Beetal and Jamnapari breeds, in Uttar Pradesh with the Barbari and Jamnapari breeds, in Bihar with the Barbari breed, in Orissa with the Beetal and Black Bengal breeds, and in Travancore-Cochin with the Malabar breed. Black Bengal is a prolific mutton breed with good scope for carcass development, while others are more valued for milk production The work has indicated the usefulness of these breeds, but in many places the schemes suffered owing to diseases and pests taking heavy toll.

Some pioneering work has been done in the Punjab to develop mohair producing quality in the local and hill goats by cross-breeding them with Angora bucks imported from South Africa. Third generation animals were the most successful mohair producers yielding 5 lbs in a year. But as the high temperature at Hissar was not found suitable for these animals, the flock has been transferred to the United Provinces Sheep and Goat Breeding Farm in Pipalkoti, Garhwal.

Sheep Breeding

Sheep breeding research received a great deal of attention during this period, and considerable progress has been made The credit goes to the Indian Council of Agricultural Research for focussing attention on the importance of the sheep industry and for initiating systematic research on sheep breeding. Mention has been made in the previous report of Hissardale sheep which was evolved at the Hissar Livestock Farm by crossing the Bikaneri sheep with the imported Merino. It was found that 7/8 Merino inheritance was suitable, and by inter-breeding the 7/8ths, a new breed called the Hissardale has been evolved which compares favourably with the Merino. Cross-breeding work in Kashmir has indicated good possibilities of developing a ¾ Merino and ¼ local Kashmir sheep which will have the approved uniformity of fine quality wool though with some shrinkage and decrease in staple length. With a view to develop the pelt industry in the N W.F. Province, the Karakul was crossed with Hashtnagri and the back-cross progeny showed good lustre and curls specially in the females In Bombay, cross-breeding work is in progress with the Merino and Deccani sheep for developing for those areas a better wool type sheep which will be free from the Deccan's black and hairy fleece.

Selective breeding in Madras, Bihar, Hissar and Poona has also yielded very good results The black faced Bellary breed of Madras is progressively improving in wool quality, and the local coloured sheep of Bihar have been losing their colour incidence in successive grades. The Bikaneri is forming out into light and medium types at Hissar, while at Bombay much improvement has been achieved in changing the Deccani sheep from black and hairy to white and woolly fleeced animals.

From the experience so far gained the following breeding policy has been adopted

(a) Selective breeding of indigenous breeds in the plains where definite breeds exist,

(b) Upgrading of nondescript with Bikaneri,

(c) Cross-breeding with foreign breeds in selected areas.

Inheritance of Mendelian characters in cattle

During the last decade, a few hereditary characters have been observed in cattle and the mode of their inheritance has been studied Schneider and Rathore (1939) studied the inheritance of white flanks in Sahiwal cattle of the Ferozepur Military Dairy Farm. The markings in some cases extended well over the flanks and in a few cases met over the back. In other animals the character was limited to the belly, flanks and lower sides. In a few animals the marking occurred as small patches only. But the pattern was always found to be bilaterally symmetrical. The character was shown to be dominant. Another study of coat colour was made by

Patel (1945) who found the red coat colour in Kankrej calves to have definite hereditary basis.

Singh and Bhattacharya (1949) found the inheritance of syndactylism in Hariana cattle of the Madhurikand Farm at Mathura to be due to autosomal recessive genes and most probably monofactorial in nature Only the fore hooves were uncleft, singly or both, and the animals became limping in gait and unsuitable for draft. This defect has also been reported by Kale (1948) in Khillari cattle The inheritance of another undesirable character 'congenital flexed fetlocks' in the Hariana herd at the Indian Veterinary Research Institute, Izatnagar, and in the Gir herd at the Indian Dairy Research Institute, Bangalore, was studied by Chandiramani and Bhattacharya (1950) who found it to be autosomal recessive in nature The defect was manifested as a sharp flexture at the distal end of the metacarpal bones and occurred in both forelegs. It acted sublethally all the calves dying within 40 days from birth.

Climate and livestock breeding

Within recent years considerable scientific study of this subject has been done abroad and it has been found that climatic factors such as air temperature and humidity play a very important part in the breeding and improvement of livestock In a subtropical country like India, which has varied climatic conditions and large number of animals, studies on the effects of climate on reproduction in farm animals becomes very necessary. The FAO conference at Lucknow in 1950 recognized this and recommended fundamental research work in this field. Mukherjee and Bhattacharya (1947 and 1948), Shukla and Bhattacharya (1947 and 1948) and Kushwaha, Mukherjee and Bhattacharya (unpublished) observed that the fertility of bulls, rams, bucks, and buffalo bulls, as judged by their semen quality, is greatly influenced by atmospheric temperatures, humidity and rainfall High air temperature associated with high humidity and rainfall had an adverse effect on their semen quality. Mukherjee and Bhattacharya (1947 and 1948) also observed that the trend of seasonal variation in haematological constituents in the males of Indian farm animals was the same as in the semen attributes. It was thus established that Indian farm animals are subject to summer subfertility associated with summer anaemia.

Artificial Insemination

Betterment of the present day degenerate livestock demands an improvement in the genetic make-up related to economic characters like milk yield, draughtability, wool, carcass quality, etc. To bring about a rapid improvement in this direction, the greatest possible use of superior sires should be made. This is possible by extensive use of artificial insemination which is the most important biological technique at present passing into general practice.

Although, a few isolated attempts had been made earlier (Kumaran 1939; Millan 1940) systematic investigation on artificial insemination with reference to Indian conditions was for the first time taken up at the Indian Veterinary Research Institute, Izatnagar, in 1942 under a scheme sponsored by the Indian Council of Agricultural Research. Studies made on the suitability of the various techniques developed abroad, on collection of semen, its preservation and insemination, demonstrated that the techniques in their broad aspects could be successfully used in Indian conditions (Bhattacharya 1946, 1949).

Collaterally with laboratory research at Izatnagar, work on artificial insemination was extended to the neighbouring villages and later at four regional centres in Calcutta, Patna, Bangalore and Montgomery The work of these centres was reviewed at the Eighth Animal Husbandry Wing Meeting at Mysore in 1949. It was considered by them that the progress made had been highly encouraging and the practicability of utilizing artificial insemination had been amply demonstrated The Montgomery Centre was closed after partition of the country in 1947.

Studies on Spermatozoa and Semen

In artificial insemination, evaluation of semen samples is of great importance for judging its fertilizing capacity There was a great paucity of knowledge on the semen characteristics of Indian farm animals and hence systematic studies were made on semen of Indian farm animals from various aspects. Shukla and Bhattacharya (1948) studied the normal semen characteristics of some breeds of Indian livestock, such as Kumauni, Sahiwal and Hariana bulls, Murrah buffaloes, goats and rams Mukherjee and Bhattacharya (1948) made similar studies with the white leghorn and indigenous poultry and found that in white leghorns, semen quality is superior to that of Desi birds Working with Indian goats, sheep and buffaloes, Mukherjee and Bhattacharya (1949) have shown that in the sexually matured animals of these species, there is a progressive morphological change leading to maturity of spermatozoa as they travel down the reproductive tract Spermatozoa when ejaculated are vigorously motile. The tail of the spermatozoon is chiefly concerned with locomotion. Its absence in mammalian spermatozoa makes them non-motile, but in avian sperm, Rao (1950) observed a peculiar worm-like passive movement even after the tail is lost. Prabhu and Guha (1950) studied the effect of muscular exercise on the quality and quantity of semen produced. With the exception of semen volume, which was higher for the non-exercised group, no significant difference was observed in the other semen characters studied. Prabhu *et al.* (1951) showed that blood meal up to the extent of 30% can be substituted in the feed concentrates of breeding bulls without impairing their semen quality and quantity. Roy *et al.* (1950) carried out studies on the correlation between the initial fructose content and rate of fructolysis in semen and other seminal attributes. They found that the rate of destruction of seminal sugar

and sperm concentration per unit volume of semen are positively correlated. The concentration of total reducing substances present in the semen of buffalo bulls, rams, goats, and cocks as well as the fructose and ascorbic acid content have been estimated by Roy *et al.* (1950) They observed that nearly 70 per cent of the reducing substances present in buffalo semen is fructose whereas in rams and goats fructose constitutes 85 per cent of the total reducing substances. This knowledge may help in developing a better semen dilutor for buffalo semen Deb (1948) devised an isothermic container for transporting preserved semen to a long distance.

Reproductive Physiology, Sex ratio, etc

Information on the normal reproductive physiological processes of Indian farm animals is lacking This information is however essential for planning any breeding programme on scientific lines Data have been collected from 10,405 animals from 46 established livestock farms (*Annu. Rep Indian vet Res Inst* 1945-49) and have been analysed Bhattacharya *et al.* (1950) studied the secondary 'sex-ratio' of normal births in Indian cattle and found that the overall sex-ratio works out to be 103.93 males to 100 female births They also found that the incidence of male births per 100 female births is 105 89 in Sahiwal, 105 80 in Sindhi, 100 41 in Tharparkar, 103.89 in Gir, 103 43 in cross-bred Sahiwal, 99 44 in cross-bred Sindhi and 111.76 in cross-bred Gir Bhattacharya *et al* (1950) also studied the incidence of abortion in Indian cattle and found that the highest proportion of abortions (25.46 per cent) occurred in second lactation after which there was a gradual fall in subsequent gestations. Bhattacharya *et al* (1950) studied the average gestation period for four pure and three grade breeds and reported the following figures (in days) · Sahiwal 285.93, Sindhi 283 88, Tharparkar 287 93, Gir 284.36, Cross-bred Sahiwal 280.65, Cross-bred Sindhi 286 37 and cross-bred Gir 283.55 They also found that overall gestation periods for male and female births varied from farm to farm and that with the exception of cross-bred Sahiwal, male gestations were slightly longer in duration than female gestations. Incidence of twinning in Indian cattle as reported by Bhattacharya *et al* (1950) is greatest in the third and fourth parturitions

HORMONES AND REPRODUCTION

Artificial lactation Luktuke and Bhattacharya (1947) induced lactation in virgin heifers and dry barren cows of Hariana breed by subcutaneous implantations and injections of diethylstilboestrol. They obtained the best results with a dry cow which gave a maximum yield of 11 lbs daily and a total of 2324 lbs. in 343 days, as compared to a total of 2910 lbs in 307 days during a previous normal lactation. In none of the treated animals was the nymphomaniac syndrome noticed Roy *et al* (1950) studied the effects of feeding thyroactive substance (Protamone) on lactation induced by stilboestrol treatment in goats. They found that the feeding of

thyroprotein after "stilboestrol induced" lactation has reached its peak brings about further increase in milk yield. Roy *et al.* (1950) experimenting on ewes produced evidence to justify the belief hitherto held that stilboestrol primarily augments andrenocorticotrophic (ACTH) secretion from the pituitary and hence the corticomimetic effects which follow stilboestrol administration.

Infertility Polding and Lall (1945) described differences in macroscopic appearance of the genitalia of the cow and the buffalo. They also studied some pathological conditions in the genitalia in these species From a study of genital organs obtained from slaughter houses, metritis was observed in 15 out of 45 and in 37 out of 185 uteri of cows and buffaloes respectively. In Indian cattle, the intercalving period is very long. With a view to shortening this, Luktuke and Bhattacharya (1948) treated a large number of cows with varying doses of pregnant mare serum gonadotrophin (PMS) They found that oestrus and ovulation were induced usually 2-4 days after treatment and most animals conceived at the induced heat Repeated inseminations in a few animals failed to produce conception though the oestrous cycle was restored in them. PMS was prepared and purified in the Division of Animal Genetics of the Indian Veterinary Research Institute and the product compared very favourably with costly commercial preparations. Several doses of the hormone prepared in the Institute have been distributed to provincial veterinary staff for trials and the reports received from them are highly satisfactory. (Bhattacharya 1951). In estimating the ascorbic acid content of ovary and corpus luteum during various stages of the oestrous cycle and early pregnancy in buffaloes, Roy *et al* (1950) observed that persistent corpus luteum, compared to normal corpus luteum is characterized by a very low concentration of ascorbic acid Freshly ovulated ovaries were found to contain a higher concentration of ascorbic acid (19.38 mg/100 g) than unovulated ones.

Bovine pregnancy diagnosis Unlike in human females and equines, the diagnosis of early pregnancy in cows and buffaloes is not possible by the known biological and chemical tests. It is, however, possible to diagnose pregnancy in cows and buffaloes from 40 days onwards or even slightly earlier by palpation of the uterus through the rectal wall This method was devised in England during the late thirties and was soon afterwards introduced in India at the Indian Veterinary Research Institute This method is widely practised now all over the country especially by the students trained in the post-graduate artificial insemination and physiopathology course conducted by the Institute.

Bhaduri and Bardhan (1949, 50) evolved a biological test using cow dung for diagnosing early bovine pregnancy. Male toads (*Bufo melanostictus*) were used as experimental animals. This test, the authors suggest, is applicable to the diagnosis of pregnancy in other farm animals also.

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